• NAAM VAN HET GENEESMIDDEL: VELCADE 3,5 mg poeder voor oplossing voor injectie. **• KWALITATIEVE EN KWANTITATIEVE SAMENSTELLING:** Elke injectieflacon bevat 3,5 mg bortezomib (als mannitolboronaatester). Na reconstitutie bevat 1 ml oplossing voor subcutane injectie 2,5 mg bortezomib. Na reconstitutie bevat 1 ml oplossing voor intraveneuze injectie 1 mg bortezomib. Hulpstoffen: Mannitol (E 421), Stikstof. **• FARMACEUTISCHE VORM:** Poeder voor oplossing voor injectie. Witte tot gebroken witte massa of poeder. **Therapeutische indicaties:** VELCADE als monotherapie is geïndiceerd voor de behandeling van volwassen patiënten met progressief multipel myeloom, die minstens 1 eerdere behandeling hebben gehad en die reeds een beenmergtransplantatie ondergaan hebben of die hiervoor niet in aanmerking komen. VELCADE in combinatie met melfalan en prednison is geïndiceerd voor de behandeling van volwassen patiënten met niet eerder behandeld multipel myeloom, die niet in aanmerking komen voor een hooggedoseerde chemotherapie met een beenmergtransplantatie. **Dosering en wijze van toediening:** De behandeling moet worden geïnitieerd en het geneesmiddel worden toegediend onder supervisie van een arts die bevoegd is tot en ervaring heeft met het gebruik van chemotherapeutica. VELCADE moet worden gereconstitueerd door een beroepsbeoefenaar in de gezondheidszorg. VELCADE 3,5 mg poeder voor oplossing voor injectie is beschikbaar voor intraveneuze of subcutane toediening. VELCADE 1 mg poeder voor oplossing voor injectie is uitsluitend beschikbaar voor intraveneuze toediening. VELCADE mag niet via andere wegen worden toegediend. Intrathecale toediening heeft geleid tot overlijden. Dosering bij monotherapie: De aanbevolen startdosering van bortezomib is 1,3 mg/m^2 lichaamsoppervlakte, twee keer per week gedurende twee weken op de dagen 1, 4, 8 en 11, gevolgd door een 10-daagse rustperiode op de dagen 12-21. Deze periode van drie weken wordt beschouwd als één behandelcyclus. Tussen opeenvolgende doses van VELCADE moet minstens 72 uur zitten. Aanbevolen wordt om patiënten met een bewezen complete remissie te behandelen met 2 additionele VELCADE-cycli na het vaststellen van de complete remissie. Ook wordt aanbevolen om responderende patiënten die geen complete remissie bereiken in totaal met 8 VELCADE-cycli te behandelen. Er zijn momenteel slechts weinig gegevens beschikbaar over herbehandeling met VELCADE. *Dosisaanpassingen tijdens behandeling en hernieuwde start van de behandeling bij monotherapie:* De VELCADE-behandeling moet worden uitgesteld als niet-hematologische graad-3-toxiciteit optreedt of hematologische graad-4-toxiciteit, met uitzondering van neuropathie, zoals hieronder wordt toegelicht. Wanneer de symptomen van de toxiciteit zijn verdwenen, mag weer met de behandeling worden begonnen, zij het met een 25% lagere dosis (1,3 mg/m^2 verlaagd tot 1,0 mg/m^2; 1,0 mg/m^2 verlaagd tot 0,7 mg/m^2). Als de toxiciteit niet verdwijnt of als de symptomen bij de laagste dosis terugkeren, moet worden overwogen met VELCADE te stoppen, tenzij het voordeel van de behandeling duidelijk opweegt tegen het risico. *Neuropathische pijn en/of perifere neuropathie:* Patiënten die bortezomib-gerelateerde neuropathische pijn en/of perifere neuropathie krijgen, moeten worden behandeld zoals aangegeven in tabel 1. Patiënten met reeds bestaande, ernstige neuropathie mogen alleen met VELCADE worden behandeld na zorgvuldige afweging van het risico en de te verwachten voordelen.

Tabel 1: Aanbevolen doseringsaanpassingen voor bortezomib-gerelateerde neuropathie*

Ernst van de neuropathie	Aanpassing van de dosering
Graad 1 (asymptomatisch; uitval van diepepeesreflexen of paresthesie) zonder pijn of functieverlies	Geen
Graad 1 met pijn of graad 2 (matige symptomen, die instrumentele activiteiten van het dagelijks leven (ADL) beperken**)	Verlaag VELCADE tot 1,0 mg/m^2 of Verander het behandelschema van VELCADE in 1,3 mg/m^2 eenmaal per week
Graad 2 met pijn of graad 3 (ernstige symptomen, die de zelfverzorgende ADL beperken***)	Onderbreek de VELCADE-behandeling tot de symptomen van de toxiciteit zijn verdwenen. Als de toxiciteit is verdwenen, opnieuw beginnen met de VELCADE-behandeling, waarbij de dosis verlaagd wordt tot 0,7 mg/m^2 één keer per week.
Graad 4 (levensbedreigende gevolgen; dringend interventie aangewezen) en/of ernstige autonome neuropathie	Stoppen met VELCADE

* Op basis van doseringsaanpassingen tijdens Fase II- en III-studies bij multipel myeloom en postmarketingervaring. Gradering gebaseerd op de NCI *Common Toxicity Criteria* CTCAE v 4.0.; ** *Instrumentele ADL (activities of daily living):* betreft bereiden van maaltijden, naar de winkel gaan voor levensmiddelen of kleding, telefoon gebruiken, geldzaken beheren, enz.; *** *Zelfverzorgende ADL:* betreft zich wassen, aan- en uitkleden, zelf eten, naar het toilet gaan, geneesmiddelen innemen en niet bedlegerig zijn.

Speciale populaties: *Leverinsufficiëntie:* Voor patiënten met lichte leverinsufficiëntie hoeft de dosis niet te worden aangepast en deze patiënten dienen met de aanbevolen dosering te worden behandeld. Patiënten met matige of ernstige leverinsufficiëntie dienen met VELCADE te starten in een verlaagde dosis van 0,7 mg/m2 per injectie tijdens de eerste behandelcyclus. Vervolgens kan een dosisverhoging tot 1,0 mg/m^2 of een verdere dosisverlaging tot 0,5 mg/m^2 worden overwogen, afhankelijk van de tolerantie bij de patiënt.

Tabel 2: Aanbevolen aanpassing van de startdosis van VELCADE bij patiënten met leverinsufficiëntie

Graad van lever-insufficiëntie*	Bilirubineconcentratie	SGOT (ASAT)-waarde	Aanpassing van de startdosis
Licht	≤ 1,0x ULN	> ULN	Geen
	> 1,0x–1,5x ULN	Elke	Geen
Matig	> 1,5x–3x ULN	Elke	Verlaag VELCADE tot 0,7 mg/m^2 in de eerste behandelcyclus. Overweeg de dosis te verhogen tot 1,0 mg/m^2 of verder te verlagen tot 0,5 mg/m^2 in de volgende cycli, afhankelijk van de tolerantie bij de patiënt.
Ernstig	> 3x ULN	Elke	

Afkortingen: SGOT = Serum-glutamaat-oxaalacetaat-transaminase; ASAT = aspartaataminotransferase; ULN = bovengrens van de normaalwaarde *(upper limit of the normal range).* *Gebaseerd op de NCI *Organ Dysfunction Working Group* classificatie voor het categoriseren van leverinsufficiëntie (licht, matig, ernstig).

Nierinsufficiëntie: De farmacokinetiek van bortezomib wordt niet beïnvloed bij patiënten met een lichte tot matige nierinsufficiëntie (creatinineklaring [CrCL] > 20 ml/min/1,73 m^2); derhalve zijn dosisaanpassingen niet nodig voor deze patiënten. Het is onbekend of de farmacokinetiek van bortezomib wordt beïnvloed bij patiënten met ernstige nierinsufficiëntie die geen dialyse ondergaan (CrCL < 20 ml/min/1,73 m^2). Aangezien dialyse de concentraties van bortezomib kan verlagen, dient VELCADE na de dialyse te worden toegediend. *Ouderen:* Er zijn geen gegevens die erop wijzen dat bij patiënten ouder dan 65 jaar dosisaanpassingen nodig zijn. *Pediatrische patiënten:* De veiligheid en de werkzaamheid van VELCADE bij kinderen jonger dan 18 jaar zijn niet vastgesteld. Dosering bij combinatietherapie: VELCADE wordt toegediend in combinatie met oraal melfalan en oraal prednison in negen behandelcycli, zoals getoond in tabel 3. Een periode van 6 weken wordt beschouwd als een behandelcyclus. In cycli 1-4 wordt VELCADE tweemaal per week toegediend op de dagen 1, 4, 8, 11, 22, 25, 29 en 32. In cycli 5-9 wordt VELCADE éénmaal per week toegediend op de dagen 1, 8, 22 en 29. Melfalan en prednison dienen beide oraal te worden gegeven op de dagen 1, 2, 3 en 4 van de eerste week van elke cyclus. Er moet minstens een tijdspanne van 72 uur zitten tussen opeenvolgende doses van VELCADE.

Tabel 3: Aanbevolen doseringsschema van VELCADE in combinatie met melfalan en prednison bij patiënten met een niet eerder behandeld multipel myeloom

VELCADE tweemaal per week (cycli 1-4)						
Week	**1**	**2**	**3**	**4**	**5**	**6**
Vc (1,3 mg/m^2)	Dag 1 -- -- Dag 4	Dag 8 Dag 11	rustperiode	Dag 22 Dag 25	Dag 29 Dag 32	rustperiode
M (9 mg/m^2) P (60 mg/m^2)	Dag 1 Dag 2 Dag 3 Dag 4	-- --	rustperiode	-- --	-- --	rustperiode
Eénmaal per week VELCADE (cycli 5-9)						
Week	**1**	**2**	**3**	**4**	**5**	**6**
Vc (1,3 mg/m^2)	Dag 1 -- -- --	Dag 8	rustperiode	Dag 22	Dag 29	rustperiode
M (9 mg/m^2) P (60 mg/m^2)	Dag 1 Dag 2 Dag 3 Dag 4	--	rustperiode	--	--	rustperiode

Vc = VELCADE; M = melfalan, P =prednison

Dosisaanpassingen tijdens behandeling en opnieuw starten van de behandeling voor combinatietherapie: Vóór het begin van een nieuwe behandelingscyclus: - het aantal plaatjes moet ≥ 70 x 10^9/l en het absolute aantal neutrofielen moet ≥ 1,0 x 10^9/l zijn; - de niet-hematologische toxiciteit moet verminderd zijn tot graad 1 of *baseline.*

Tabel 4: Wijziging van de dosering in opeenvolgende cycli

Toxiciteit	Wijziging van de dosering of uitstel van de toediening
Hematologische toxiciteit in een cyclus: • Indien langdurige neutropenie of trombocytopenie van graad 4 of trombocytopenie met bloeding werd waargenomen in de vorige cyclus	Overweeg vermindering van de dosis melfalan met 25% in de volgende cyclus.
• Indien aantal plaatjes ≤ 30 × 10^9/l of ANC ≤ 0,75 x 10^9/l op een dag waarop VELCADE wordt toegediend (behalve dag 1)	De VELCADE-behandeling niet geven
• Indien verschillende dosissen van VELCADE in een cyclus achterwege blijven (≥ 3 dosissen bij toediening tweemaal per week of ≥ 2 dosissen bij toediening éénmaal per week)	De dosis VELCADE moet verminderd worden met één dosisniveau (van 1,3 mg/m^2 tot 1 mg/m^2, of van 1 mg/m^2 tot 0,7 mg/m^2)
Niet-hematologische toxiciteit van graad ≥ 3	De behandeling met VELCADE moet onderbroken worden tot de symptomen of de toxiciteit verminderd zijn tot graad 1 of *baseline.* VELCADE mag dan opnieuw toegediend worden met één niveau van dosisvermindering (van 1,3 mg/m^2 tot 1 mg/m^2, of van 1 mg/m^2 tot 0,7 mg/m^2). Bij VELCADE-gerelateerde neuropathische pijn en/of perifere neuropathie, moet de dosis VELCADE onderbroken en/of gewijzigd worden zoals aangegeven in Tabel 1.

Voor bijkomende informatie met betrekking tot melfalan en prednison, zie de betreffende Samenvatting van de Productkenmerken.

Wijze van toediening: *Intraveneuze injectie:* VELCADE 3,5 mg gereconstitueerde oplossing wordt toegediend als een intraveneuze bolusinjectie (3-5 seconden) via een perifere of centrale intraveneuze katheter, gevolgd door een spoeling met 9 mg/ml (0,9%) natriumchlorideoplossing voor injectie. Er moet minstens een tijdspanne van 72 uur zitten tussen opeenvolgende doses van VELCADE. *Subcutane injectie:* VELCADE 3,5 mg gereconstitueerde oplossing wordt subcutaan toegediend in de dij (rechts of links) of de buik (rechts of links). De oplossing moet subcutaan worden geïnjecteerd, onder een hoek van 45-90°. De injectieplaatsen moeten worden afgewisseld voor achtereenvolgende injecties. Als er lokale reacties op de injectieplaats optreden na een subcutane injectie met VELCADE, kan ofwel een minder geconcentreerde VELCADE oplossing subcutaan worden toegediend (VELCADE 3,5 mg gereconstitueerd tot 1 mg/ml in plaats van 2,5 mg/ml) of wordt aanbevolen over te schakelen op intraveneuze injectie. **Contra-indicaties:** Overgevoeligheid voor bortezomib, borium of een van de hulpstoffen. Acute diffuse infiltratieve pulmonale en pericardiale aandoening. **Bijwerkingen:** *Samenvatting van het veiligheidsprofiel:* De bijwerkingen die tijdens behandeling met VELCADE het meest werden gemeld, zijn nausea, diarree, obstipatie, braken, vermoeidheid, pyrexie, trombocytopenie, anemie, neutropenie, perifere neuropathie (waaronder sensorische), hoofdpijn, paresthesie, verminderde eetlust, dyspneu, huiduitslag, herpes zoster en myalgie. Ernstige bijwerkingen die tijdens behandeling met VELCADE soms werden gemeld, zijn hartfalen, tumorlysissyndroom, pulmonale hypertensie, PRES, acute diffuse infiltratieve longaandoeningen en zelden autonome neuropathie. *Samenvattende tabel met bijwerkingen:* De bijwerkingen in tabel 5 hebben volgens de onderzoekers minstens een mogelijke of waarschijnlijke causale relatie met VELCADE. Deze bijwerkingen zijn gebaseerd op een integraal gegevensbestand van 3.628 patiënten van wie 2.606 patiënten werden behandeld met VELCADE in een dosis van 1,3 mg/m^2. Onder deze 2.606 patiënten bevonden zich: 2.068 patiënten met multipel myeloom die VELCADE intraveneus toegediend kregen als monotherapie, 369 patiënten met multipel myeloom die VELCADE intraveneus toegediend kregen in combinatie met melfalan en prednison, 147 patiënten met multipel myeloom die VELCADE subcutaan toegediend kregen als monotherapie en 22 patiënten met chronische lymfatische B-celleukemie (CLL) die VELCADE intraveneus toegediend kregen als monotherapie. In totaal werd VELCADE voor de behandeling van multipel myeloom toegediend aan 2.584 patiënten. De bijwerkingen worden hieronder per orgaansysteem en frequentie vermeld. De frequentie wordt gedefinieerd als: zeer vaak (≥ 1/10); vaak (≥ 1/100 tot < 1/10); soms (≥ 1/1.000 tot < 1/100); zelden (≥ 1/10.000 tot < 1/1.000); zeer zelden (< 1/10.000), niet bekend (kan met de beschikbare gegevens niet worden bepaald). Binnen elke frequentiegroep zijn de bijwerkingen aangegeven in orde van afnemende ernst. Tabel 5 is gegenereerd met gebruik van versie 13.1 van de MedDRA. Postmarketing-bijwerkingen die niet werden gezien in klinische studies zijn ook toegevoegd. *Tabel 5: Bijwerkingen bij patiënten behandeld met VELCADE als monotherapie of in combinatie* **(Systeem/orgaanklasse**, Incidentie, Bijwerking*):* **Infecties en parasitaire aandoeningen:** Vaak: herpes zoster (incl. verspreid & oftalmisch), pneumonie*, infectie*, herpes simplex*, schimmelinfectie*; Soms: sepsis*, bronchopneumonie, herpesvirusinfectie*, bacteriëmie (incl. stafylokokken), hordeolum, griep, cellulitis, hulpmiddelgerelateerde infectie, huidinfectie*, oorinfectie*, tandinfectie*; Zelden: meningitis (incl. bacterieel), Epstein-Barr-virusinfectie, erysipelas, herpes genitalis, stafylokokken-infectie, tonsillitis, varicella, mastoïditis, postvirale vermoeidheidssyndroom. **Neoplasmata, benigne, maligne en niet-gespecificeerd (inclusief cysten en poliepen):** Soms: neoplasma maligne; Zelden: plasmacytaire leukemie, niercel carcinoom, gezwel, mycosis fungoides, neoplasma benigne. **Bloed- en lymfestelsel-aandoeningen:** Zeer vaak: trombocytopenie*, neutropenie*, anemie*, leukopenie*; Vaak: lymfopenie*; Soms: pancytopenie*, febriele neutropenie, coagulopathie*, leukocytose*, lymfadenopathie; Zelden: hyperviscositeitssyndroom, trombocytopenische purpura*, bloedziekte NAO, hemorragische diathese, lymfocytaire infiltratie. **Immuunsysteem-aandoeningen:** Soms: overgevoeligheid*; Zelden: anafylactische shock, immuuncomplex gemedieerde reactie type III. **Endocriene aandoeningen:** Soms: hyperthyreoïdie*, antidiuretisch hormoon-secretiedeficiëntie; Zelden: Cushing-syndroom*, hypothyreoïdie. **Voedings- en stofwisselings-stoornissen:** Zeer vaak: verminderde eetlust; Vaak: electrolytevenwichtstoornis*, dehydratie, enzymafwijking*, hyperurikemie*; Soms: tumorlysissyndroom, niet goed groeien*, hypoglykemie*, hyperglykemie, hypoproteïnemie*, vochtretentie, hypovolemie; Zelden: acidose, vochtopstapeling, hypocholremie*, Diabetes mellitus*, hyperproteïnemie*, hypo-urikemie*, metabole stoornis, vitamine-B-complex-deficiëntie, vitamine-B12-deficiëntie, jicht, hyperammoniëmie*, verhoogde eetlust, alcoholintolerantie. **Psychische stoornissen:** Vaak: stemming veranderd*, angststoornis*, slaapstoornis*; Soms: psychische stoornis*, hallucinatie*, verwarring*, rusteloosheid; Zelden: zelfmoordgedachte*, psychotische stoornis*, abnormale dromen, aanpassingsstoornis, delirium, verminderd libido. **Zenuwstelsel-aandoeningen:** Zeer vaak: perifere neuropathie*, perifere sensorische neuropathie, dysesthesie*, neuralgie*, hoofdpijn*; Vaak: perifere motorische neuropathie, bewustzijnsverlies (incl. syncope), duizeligheid*, dysgeusie*, lethargie; Soms: intracraniale bloeding*, tremor, perifere sensimotorische neuropathie, ataxie*, dyskinesie*, geheugen vermindering*, encefalopathie*, evenwichtsstoornis, neurotoxiciteit, presyncope, postherpetische neuralgie, spraakstoornis*, restless legs-syndroom, migraine, ischias, stoornis van aandacht, reflexen afwijkend*, parosmie; Zelden: hersenoedeem, cerebrale hemorragie, transient ischaemic attack, autonoom zenuwstelsel stoornis, autonome neuropathie, convulsie, craniale verlamming*, paralyse*, parese*, hersenstamsyndroom, cerebrovasculaire aandoening, zenuwwortellaesie, psychomotorische hyperactiviteit, ruggenmerg compressie, cognitieve aandoening NAO, motore disfunctie, zenuwstelselaandoening NAO, radiculitis, kwijlen, hypotonie. **Oogaandoeningen:** Vaak: oogzwelling*, gezichtsvermogen afwijkend *, conjunctivitis*, droog oog*; Soms: oogbloeding*, ooglidinfectie*, oogontsteking*, oculaire hyperemie, diplopie, oogirritatie*, oogpijn, traanproductie verhoogd, oogafscheiding; Zelden: cornealetsel*, exoftalmie, retinitis, scotoma, oogaandoening (incl. ooglid) NAO, dacryoadenitis verworven, fotofobie, fotopsie, optische neuropathie#, verschillende graden van verminderd gezichtsvermogen (t/m blindheid)*. **Evenwichtsorgaan- en ooraandoeningen:** Vaak: vertigo*; Soms: gehoor beschadigd (t/m doofheid), dysacusis*, tinnitus*, oorongemak*; Zelden: oorbloeding, ooraandoening NAO. **Hartaandoeningen:** Vaak: hartfalen*, tachycardie*; Soms: cardiopulmonaire stilstand*, hartfibrilleren (incl. atrium), palpitaties, angina pectoris, pericarditis*, cardiomyopathie*, ventriculaire dysfunctie*, bradycardie; Zelden: atriale flutter, myocardinfarct*, atrioventriculair blok*, hart- en vaataandoening (incl. cardiogene shock), torsade de pointes, angina instabiel, kransslagaderinsufficiëntie, linkerventrikelfalen, mitralisklepinsufficiëntie, sinusstilstand. **Bloedvat-aandoeningen:** Vaak: hypotensie*, orthostatische hypotensie, hypertensie*; Soms: diepe veneuze trombose*, hemorragie*, tromboflebitis (incl. oppervlakkige), circulatoire collapse (incl. hypovolemische shock), flebitis, overmatig blozen, hematoom*, slechte perifere circulatie*, opvlieger, vasculitis, bleekheid; Zelden: perifere embolie, lymfoedeem, erythromelalgia, vasodilatatie, verkleurde vene, veneuze insufficiëntie. **Ademhalings-stelsel-, borstkas- en mediastinum-aandoeningen:** Vaak: dyspneu*, bloedneus, bovenste/onderste luchtweginfectie*, hoesten*; Soms: longembolie, pleurale effusie, pulmonaal oedeem (incl. acuut), bronchospasme, chronisch aspecifieke respiratoire aandoening*, hypoxemie*, pulmonaire hypertensie, luchtwegcongestie*, hypoxie, pleura-ontsteking*, longfibrose, hik, rhinorroe, dysfonie, piepen; Zelden: respiratoir falen, acuut ademhalingsnoodsyndroom, apneu, pneumothorax, atelectase, haemoptysis, hyperventilatie, orthopnoea, pneumonitis, respiratoire alkalose, tachypnoea, hypocapnie*, interstitiële longziekte, longinfiltraat, keelbeklemming, droge keel, bronchiale hyperreactiviteit, toegenomen luchtweguitscheiding, keelirritatie. **Maagdarmstelsel-aandoeningen:** Zeer vaak: braken, diarree*, nausea, obstipatie, abdominale pijn (incl. gastro-intestinale pijn)*; Vaak: gastro-intestinale bloeding (incl. mucosa)*, dyspepsie, stomatitis*, abdominale distensie, orofaryngeale pijn*, abdominaal ongemak, mondaandoening*, flatulentie; Soms: pancreatitis (incl. chronisch), hematemesis, lipzwelling*, orale ulceratie*, ileus*, enteritis*, gastritis*, gingiva-bloeding, gastro-oesofageale refluxziekte*, maagdarmontsteking*, dysfagie, prikkelbare darmsyndroom, oesofagitis, gastro-intestinale aandoening NAO, braakneiging, maagdarmstelselmotiliteitsaandoening*, speekselklieraandoening*, orofaryngeale blaarvorming*; Zelden: pancreatitis acuut, peritonitis*, tongoedeem*, ascites, cheilitis, fecale incontinentie, anussfincteratonie, fecaloom, rectale afscheiding, lippijn, periodontitis, anale fissuur, wijziging in darmgewoonte, proctalgie, abnormale feces. **Lever- en galaandoeningen:** Vaak: leverenzym abnormaal*; Soms: hepatotoxiciteit (incl. leveraandoening), hepatitis*, cholestase; Zelden: leverfalen, hepatomegalie, Budd-Chiari-syndroom, leverbloeding, cholelithiase. **Huid- en onderhuid-aandoeningen:** Zeer vaak: rash*; Vaak: urticaria, pruritus*, erytheem, dermatitis*, droge huid; Soms: acute febriele neutrofiele dermatose, toxische huideruptie, haaraandoening*, petechiae, ecchymose, huidlaesie, purpura, huidknobbel*, psoriasis, palmoplantair erytrodysesthesiesyndroom, hyperhidrose, nachtzweet, acne*, bloedblaar, pigmentatieaandoening*, nagelafwijking; Zelden: erythema multiforme, huidreactie, Jessner's lymfocytaire infiltratie, subcutane bloeding, livedo reticularis, huidverharding, blaar, koud zweet, papel, fotosensitiviteitsreactie, seborrhoea, huidaandoening NAO. **Skeletspierstelsel- en bindweefsel-aandoeningen:** Zeer vaak: skeletspierstelselpijn*; Vaak: spierspasmen*, pijn in extremiteiten, spierzwakte; Soms: spiertrekkingen, gewrichtszwelling, artritis*, gewrichtsstijfheid, myopathie*, zwaar gevoel; Zelden: rabdomyolyse, temporomandibulair gewrichtssyndroom, fistel, vochtophoping in gewricht, pijn in kaak, botaandoening, dactylitis, synoviumcyste. **Nier- en urineweg-aandoeningen:** Vaak: nierfunctie verminderd*, chronisch nierfalen*; Soms: acuut nierfalen, urineweginfectie*, hematurie*, urineretentie, dysurie*, urinelozingsaandoening*, proteïnurie, azotemie, oliguria*, pollakisurie; Zelden: nierkoliek, blaasprikkeling, urinegeur abnormaal. **Voortplantings-stelsel- en borstaandoeningen:** Soms: vaginale bloeding, geslachtsorgaanpijn*, erectiele disfunctie, testiculaire aandoening*; Zelden: prostatitis, borstaandoening vrouwelijk, epididymisgevoeligheid, epididymitis, bekkenpijn, vulvale ulceratie. **Congenitale, familiale en genetische aandoeningen:** Zelden: aplasie, maagdarmstelselmisvorming, ichthyose. **Algemene aandoeningen en toedieningsplaats-stoornissen:** Zeer vaak: pyrexie*, vermoeidheid, asthenie; Vaak: oedeem (incl. perifeer), koude rillingen, pijn*, injectieplaatsreactie*, malaise*; Soms: dood (incl. plotse), algemene deterioratie van lichamelijke gezondheid*, gezichtsoedeem*, borstkaspijn, slijmvliesaandoening*, loopstoornis, het koud hebben, extravasatie*, kathetergerelateerde complicatie*, dorst, borstkasongemak, gevoel van verandering in lichaamstemperatuur*, injectieplaatspijn*; Zelden: bloeding injectieplaats*, hernia*, injectieplaatsflebitis*, afgenomen genezing, ontsteking, gevoeligheid, ulcus, prikkelbaarheid, niet-cardiale pijn op de borst, pijn op de katheterplaats, gevoel van vreemd lichaam. **Onderzoeken:** Vaak: gewicht verlaagd; Soms: hyperbilirubinemie*, gewicht verhoogd, C-reactief proteïne verhoogd; Zelden: megakaryocyten verlaagd, pO2 verhoogd, bloed bicarbonaat verlaagd, bloed creatinine verhoogd*, elektrocardiogram abnormaal*, internationale genormaliseerde ratio abnormaal*, bèta-2-microglobuline verhoogd, bloed creatinine verlaagd, cytomegalievirustest, maag pH verlaagd, plaatjesaggregatie verhoogd, troponine I verhoogd, bloed testosteron verlaagd, eiwit urine aanwezig, serum ferritine verhoogd, pH urine verhoogd. **Letsels, intoxicaties en verrichtingscom-plicaties:** Soms: vallen, kneuzing; Zelden: schedelbreuk, transfusiereactie, rigors*, aangezichtsletsel, gewrichtsletsel, inscheuring, procedurepijn, bestralingsletsels*. **Chirurgische en medische verrichtingen:** Zelden: macrofagenactivatie. (NAO = niet anders omschreven; *Groepering van meer dan één MedDRA-voorkeursterm; # Postmarketing-bijwerking). Beschrijving van bepaalde bijwerkingen: *Reactivatie van het herpes zoster-virus:* Antivirale profylaxe werd toegediend aan 26% van de patiënten in de Vc+M+P-arm. De incidentie van herpes zoster bij patiënten in de groep behandeld met Vc+M+P was 17% voor patiënten die geen antivirale profylaxe kregen toegediend, tegenover 3% voor patiënten die wel antivirale profylaxe kregen toegediend. *Opvallende verschillen in het veiligheidsprofiel van VELCADE subcutaan toegediend, versus intraveneus toegediend als monotherapie:* Patiënten die VELCADE in de Fase III studie subcutaan kregen hadden in vergelijking met intraveneuze toediening een 13% lagere totale incidentie van bijwerkingen met een toxiciteit van graad 3 of hoger ontstaan na het begin van de behandeling en een 5% lagere incidentie van het stoppen met VELCADE. De totale incidentie van diarree, gastro-intestinale en abdominale pijn, asthenie-aandoeningen, infecties van de bovenste luchtwegen en perifere neuropathieën waren in de groep met subcutane toediening 12%-15% lager dan in de groep met intraveneuze toediening. Bovendien was de incidentie van perifere neuropathieën van graad 3 of hoger 10% lager en het percentage dat vanwege perifere neuropathie stopte 8% lager voor de subcutane groep dan voor de intraveneuze groep. Van zes procent van de patiënten werd gemeld dat ze een lokale bijwerking op de subcutane toediening hadden, meestal roodheid. Deze gevallen gingen in een mediane tijd van 6 dagen over; bij twee patiënten was aanpassing van de dosis nodig. Twee patiënten (1%) hadden ernstige reacties; 1 geval van pruritus en 1 geval van roodheid. De incidentie van overlijden tijdens de behandeling was 5% in de subcutane behandelgroep en 7% in de intraveneuze behandelgroep. Incidentie van overlijden door 'progressieve ziekte' was 18% in de subcutane groep en 9% in de intraveneuze groep. **Aard en inhoud van de verpakking:** Type I glazen injectieflacon van 10 ml met een grijze broombutylstop en een aluminiumafsluiting, met een koningsblauwe dop, met 3,5 mg bortezomib. De injectieflacon is verpakt in een doorzichtige blisterverpakking die bestaat uit een schaaltje met een deksel. Elke verpakking bevat 1 injectieflacon voor eenmalig gebruik. **• HOUDER VAN DE VERGUNNING VOOR HET IN DE HANDEL BRENGEN:** JANSSEN-CILAG INTERNATIONAL NV, Turnhoutseweg 30, B-2340 Beerse, België. **• NUMMER VAN DE VERGUNNING VOOR HET IN DE HANDEL BRENGEN:** EU/1/04/274/001. **• AFLEVERINGSWIJZE:** Geneesmiddel op medisch voorschrft. **• DATUM VAN HERZIENING VAN DE TEKST:** 20/09/2012. *Meer informatie is beschikbaar op verzoek.*

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INTRODUCTION

The Belgian Hematological Society is happy to welcome you to its 28th General Annual Meeting (GAM), in the International Congress Center (ICC) in Gent!

This year the meeting will be held for three days (Thursday through Saturday morning) instead of the usual two.

The GAM is a unique opportunity for scientists and clinicians interested in haematology research and in the care of patients affected with blood diseases to meet colleagues and friends, to attend state-of-the art presentations and to share ongoing laboratory and clinical research activities. This is a great opportunity for interaction and continuing education.

As usual, the GAM will feature:
- lectures by a distinguished panel of Belgian and foreign speakers on topics relating to malignant and non-malignant haematology (see detailed program);
- oral presentations selected from submitted abstracts
- poster walks: posters are divided into four broad topics, with a chairman for each topic conducting short presentations by authors followed by a discussion with the audience;
- satellite symposia: one sponsored by Janssen on novel agents and a second one by Celgene on imids in haematological malignancies.

In addition to the main meeting, a number of important parallel sessions will be organised:
- on Thursday afternoon, we will have a session organised by the BHS committees to present their burgeoning activities to a larger audience and to discuss new projects;
- on Thursday afternoon also, the regulatory affairs committee is organising its very successful National JACIE meeting day to discuss new development in the regulation and accreditation of haematopoietic cell transplantation;
- on Friday afternoon, there will be a parallel session organised by the ABCA / BVAC association on flow cytometry, an excellent opportunity for our two organisations to meet and collaborate; a satellite symposium sponsored by Alexion will focus on deficiencies of complement regulators.

Finally, important sessions will be organised for nurses and patients:
- the nurses committee is extending its annual meeting to a full day meeting on Friday;
- last but not least, we are organising for the first time a BHS patient day, and we have chosen the theme of myelodysplactic syndromes for this year, but the topic will change every year.

We will again conduct a truly interactive business meeting in the middle of the Saturday morning session and we would really like to encourage participants in the BHS meeting to attend. The business meeting will be devoted to:
- presentations by the board of its accomplishments and ongoing projects;
- election of a new President, vice-president and board of the BHS for 2013-2015;

Indeed, the board of the BHS is thrilled to share with you its projects for the society.

Yves Beguin
President of the BHS

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10.00 Treatment of low-risk MDS
V. Robin (Mons)
10.30 Treatment of high-risk MDS
D. Selleslag (Brugge)

11.00 Coffee break

11.30 - 12.00 **Patient experience with myelodysplastic syndromes**
Moderators : V. Prockl & F. Mainil

11.30 Testimony by French-speaking patient
11.45 Testimony by Dutch-speaking patient

12.00 - 12.30 **Life with a myelodysplastic syndrome**
Moderators : A, Coolbrant & P. Crombez

Panel (D. Selleslag, V Robin, V. Prockl, the 2 patients) discussion with MDS patients about their experience with the disease and quality of life.

12.30 - 13.15 **A MDS patient group**
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K. Theunissen (Hasselt)
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Moderators : M. Quaghebeur & G. Mertens

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A. Vannucchi (Florence, Italy) (supported by an unrestricted grant from Novartis)

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M. Lemaire (VUB)
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Angiogenesis, also in leukaemia
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Programme BHS General Annual Meeting — Saturday 26 January 2013

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Chairmen: Valerie Robin, Eric Vandenneste

09.00 Biology
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11.30 Update on the clinical use of Imids in haemato-oncology
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12.00 Mechanism of action of IMIDs and implications for future therapeutic approaches
R. Chopra (Celgene)

12.30 Conclusion, awards & lunch

Abstracts state of the art and special highlights lectures

Therapeutic approach to myelofibrosis in the JAK2 inhibitor era

Alessandro M. Vannucchi, University of Florence, Italy

Among chronic myeloproliferative neoplasms (MPN), primary myelofibrosis has the most adverse outcome with reduced survival compared to control population; although less known, also myelofibrosis that arises after polycythemia vera or essential thrombocythemia have a similar course. Risk stratification systems have been developed that allow to identify subjects at higher risk of death in whom stem cell transplant (SCT), the only curative approach to date, is appropriate. These include the International Prognostic Scoring System (IPSS), developed by the IWG-MRT, based on five clinical and hematologic variables estimated at the time of diagnosis (age >65 years, hemoglobin level <100 g/L, leukocyte count >25x109/L, presence of at least 1% blasts in the peripheral blood and complaint of constitutional symptoms; the Dynamic IPSS, that has the advantage to make survival estimates feasible at any time during disease course using the same set of variables; and the DIPPS-plus, that incorporates three additional variables for improved discrimination of patients' risk categories (red cell transfusion need, not treatment-related thrombocytopenia and unfavorable karyotype (ie: complex karyotype or sole or two abnormalities such as +8, -7/7q-, i(17q), inv(3), -5/5q-, 12p-, 11q23)). Based on the scores, four different risk categories (low, intermediate-1 and -2, high-risk) are identified with survival ranges from two years to greater than ten years. However, while these scores are very useful for selecting patients for SCT and for making prognostication, they are not of practical usefulness for conventional treatment choices that largely depend on the protean presentation of disease. The most frequent reasons why patients with MF need treatment are represented by anemia, splenomegaly and/or hepatomegaly, symptomatic foci of non hepatosplenic haematopoiesis, uncontrolled leukocytosis or thrombocytosis, thrombo-hemorrhagic complications and severe, debilitating constitutional symptoms. On the other hand, patients who have no or minimal symptoms can be managed with a 'watch-and-wait' strategy, postponing a therapeutic decision until the appearance of hematologic abnormalities or worsening of clinical manifestations. Modest and transient improvements, if at all, can be obtained with conventional therapy, therefore appropriate treatment of subjects with MF remains largely unmet clinical need. This scenario has changed abruptly after the introduction of JAK2 inhibitors, finally resulting in the approval of the first drug for myelofibrosis, Ruxolitinib, by FDA in November 2011, followed by approval from Canafian and European Agencies, at this time. The approval was granted following the results of two large phase III trials, COMFORT-I and –II, comparing Ruxolitinib with placebo and best-available therapy (BAT, respectively. In both studies, Ruxolitinnib produced significant improvements in splenomegaly (primary end point was a reduction of at least 35% by MRI of spleen volume, equaling a at least 50% reduction of palpatory spleen) and in constitutional symptoms. The treatment was well tolerated, with few and minor non-haematologic toxicities. Main toxicity was thrombocytopenia (a DLT) and anemia, manageable with blood transfusions usually for some months after beginning of treatment. At more than two years after trial initiation, Ruxolitinib continues to be well tolerated, a mean duration of spleen response has not been reached yet. Retrospective comparison against characteristics-matched patients of the subjects enrolled in the phase 2 trial with Ruxolitinib suggested an improvement of survival, especially for the category at high-risk, that was also dependent on the achievement of the primary end point for spleen reduction. However, a retrospective analysis from a single institution (Mayo Clinic) did not confirm the survival advantage. Preliminary data from both COMFORT trials, although they were not powered to detect differences in survival, appear to confirm some advantage for patients receiving Ruxolitinib compared to both placebo and BAT. Finally, analysis of molecular response showed that a subgroup of 25% of the patients presented appreciable reductions of the JAK2V617F allele burden that were, in turn, associated with the greatest spleen volume responses. Since Ruxolitinib, as well as other JAK2 inhibitors in use, is not specific for the mutation JAK2, it resulted similarly effective in both JAK2V617F and JAK2 wild-type patients, and also irrespective of starting spleen volume, age, risk category, and type of MF.

Other JAK2 inhibitors are under evaluation in clinical trials. The most advanced include SAR2305, that has completed a phase III trial (JAKARTA; results pending), CYT387 (that has been reported to produce responses of anemia in more than one third of the patients) and LY2784544. Furthermore, combination studies that include Ruxolitinib and another drug, such as panobinostat (an HDAC inhibitor) or a PI3K inhibitor (BMK120), are ongoing. Also, the better position of Ruxolitinib in the settings of stem cell transplantation is being explored.

From being neglected disorders, PMF and the other MPNs have become an active field of research, holding the promise to improve the management of patients.

Angiogenesis in Hematology: from basic science to clinic

Peter Carmeliet, VIB-KU Leuven, Belgium

Angiogenesis, the growth of new blood vessels, plays a crucial role in numerous diseases, including cancer. Anti-angiogenesis therapies have been developed to deprive the tumour of nutrients. Clinically approved anti-angiogenic drugs offered prolonged survival to numerous cancer patients. However, the success of anti-angiogenic VEGF-targeted therapy is limited in certain cases by intrinsic refractoriness and acquired resistance. New strategies are needed to block tumour angiogenesis via alternative mechanisms. We are therefore exploring whether targeting endothelial metabolism can be a possible alternative therapeutic strategy for anti-angiogenic therapy.

Epigenetic regulation of hematological malignancies

Elke De Bruyne, University of Brussels, Belgium

The field of epigenetics consists of all heritable changes of the transcriptome without altering the primary DNA sequence. DNA methylation of cytosine bases within a CpG dinucleotide and post-translational histone modifications (acetylation, methylation, phosphorylation, ubiquitination) represent the two best documented epigenetic modifications and are broadly acknowledged to co-operate intensively to control the organization of the chromatin architecture and hence transcriptional regulation. Generally, DNA methylation is repressive for transcription, while histone modifications can be both active or repressive depending on the type, degree and the site. In mammalians, CpG-rich regions (CpG islands) located at the intergenic regions are heavily methylated in normal cells and are associated with repressive histone marks thus governing genomic stability. In contrast, most of the promoter associated CpGs islands are unmethylated and associated with permissive histone marks thus permitting gene expression, except for tissue

specific genes or genes involved in X-chromosome inactivation and imprinting. Consequently, epigenetic modifications play a crucial role in normal development and are essential for maintaining cell identity and normal functionality. In most cancers, including myeloid and lymphoid malignancies, the epigenetic landscape is completely disrupted. Intergenic regions have become severely hypomethylated and associated with active PTM marks leading to genomic instability, while the promoter-associated CpG islands of tumor suppressor genes are often hypermethylated and associated with repressive PTM marks resulting in loss-of-function. The fundamental difference between epigenetic and genetic alterations is the reversible nature of the former, making chromatin-modifying enzymes, referred to as epigenetic 'writers' and 'erasers', today one of the most promising and pursued drug targets. Already, inhibitors of histone deacetylases (HDACi; panobinostat, vorinostat) and DNA methyltransferases (DNMTi; azacytidine and decitabine) have demonstrated substantial clinical efficacy in haematological malignancies (especially in MDS, AML and CML, but also in MM). Nevertheless, despite these encouraging preliminary results, to date single-agent anti-tumour effects of these epigenetic modulating agents are only modest in most haematological malignancies. This can, in part, be explained by the fact that the pleiotropic mechanisms by which the epigenetic-modulating agents mediate their anti-tumour activity, especially *in vivo*, remain largely unknown. An interesting and complex issue is to what extent the neoplastic cells are epigenetically modified after treatment *in vivo* where the cells are protected by the surrounding cells of the tumour microenvironment. Consequently, these agents remain under intensive investigation and pre(clinical) studies are ongoing to investigate new dosing schedules, routes of administration and combination with other (chemotherapeutic) agents. In the first part of the presentation an overview of the commonly observed epigenetic aberrations in haematological malignancies and the obtained results of clinical trials will be given. In a second part, we then discuss molecular events underlining the antitumor effects of HDACi and DNMTi and strategies to improve responsiveness to epigenetic modulating drugs using MM as a model for epigenetic dysregulation in hematological malignancies.

Regulatory T cells: from bench to bedside

Stéphanie Humblet-Baron, University of Leuven, Belgium

Although the concept of an immunosuppressive T cell population has been suggested in the 1970s, it is only in the late 1990s that a distinct T cell entity with immunosuppressive properties has been described and termed regulatory T cell (Treg). Later, a specific molecule, the transcription factor FoxP3 (Forkhead box P3), has been identified as the hallmark of Treg, and has been shown to confer to Treg their suppressive function. In the clinics, the critical role of Treg has been demonstrated by the observation that patients lacking FoxP3 function develop the IPEX syndrome, a severe x-linked auto-immune syndrome combining immune dysregulation, polyendocrinopathy, and enteropathy.

Because of their major suppressive functions, investigators have raised the hope that Treg infusion could be useful in the treatment of severe uncontrolled inflammation in different clinical conditions. Based on encouraging results in animal models of allogeneic hematopoietic stem cell transplantation showing that Treg infusion could prevent graft-versus-host disease (GVHD, a redoubtable complication of allogeneic hematopoietic cell transplantation consisting of destruction of host organs by donor immune cells contained in the graft), Treg infusions have been first assessed (and proved useful) as GVHD prevention in patients given allogeneic hematopoietic stem cells from HLA-mismatched donors. Further, interleukin-2 mediated Treg expansion has been demonstrated as an useful tool in patients suffering from severe corticosteroids-refractory chronic GVHD. In this lecture, recent advances in Treg development, mechanism of suppression and homeostasis will be presented as well as their implications in different primary immunodeficiency disorders. Finally, the hopes and limitations of Treg infusion / in-vivo expansion in the setting of allogeneic hematopoietic stem cell transplantation will be discussed.

Macrophage activation syndromes

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Haemophagocytic lymphohistiocytosis (HLH) is a rare life-threatening disease of immune dysregulation, a syndromic disorder defined by a unique pattern of clinical and laboratory findings. Cytotoxic T- and NK-cells and macrophages cause multi-organ damage, haemophagocytosis, secrete high amounts of inflammatory cytokines that are responsible for a severe systemic inflammation. The first episode of HLH can occur from prenatal presentations through the seventh-eight decades, and the clinical spectrum ranges from mild and self-limited HLH to rapidly fatal multi-organ failure. Genetic or primary HLH clearly belongs to primary immunodeficiency diseases. Acquired HLH is associated with infectious agents, autoimmune or autoinflammatory diseases (macrophage activation syndrome), malignant diseases, or any acquired immune deficiency. Early recognition of this syndrome and rapid therapeutic intervention are of critical importance. I will focus on major questions regarding diagnosis and treatment in adult HLH.

Chronic lymphocytic leukemia – biology

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The last decade has seen an amazing advancement in our knowledge of the biology and natural history of Chronic Lymphocytic Leukemia (CLL). Several evidences strongly support a crucial role of micro-environmental signals in the onset and progression of the disease. These include both soluble and membrane-bound interactions occurring between the leukemic cells and the surrounding by-standers cells as T cells or stromal cells. One of the key elements that attracted the attention of the investigators has been the antigenic stimulation though the Immunoglobulin receptor on the surface of the CLL B cells. This interest originated from the seminal observation that the presence or the absence of somatic mutations in the leukemic Ig associated with the clinical outcome of patients. More importantly, this notion, together with many other evidences, led to the concept that indeed all CLL cases should be considered antigen-experienced B cells. The nature of the antigens involved in the leukemogenic process have been reported, including self-antigens present in the apoptotic bodies. The encounter with the antigens may produce different if not opposite fates of the leukemic clone with some de-energized by the stimulation while others being activated. This dichotomy somehow mirrors the opposite clinical course (indolent versus aggressive) that can be observed among CLL patients . All this information has now been comprised into the very exciting possibility of interfering with the signaling pathways originating from the B cell antigen receptor, using targeted therapies, that are now under investigation in clinical trials. Once more, it shows that the understanding of the molecular events occurring during the natural history of a disease, offers the best opportunity not only to improve our scientific knowledge but also to improve the available therapies and to design tailored treatments.

Treatment for Chronic Lymphocytic Leukemia

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Chronic lymphocytic leukaemia (CLL) is a clonal lymphoid disease characterised by the proliferation and accumulation of small CD5/CD19-positive lymphocytes in the blood, lymph nodes, spleen, liver and bone marrow.[1] CLL is the most common indolent lymphoid leukaemia, accounting for approximately 30% of all leukaemia in Europe and North America, with an estimated annual age-adjusted incidence of three to five per 100 000 persons.[2] The disease is diagnosed most commonly in the elderly, with the median age at diagnosis 65–70 years and 80% of patients diagnosed being over 60 years of age.[3]

The natural clinical course of CLL is highly variable. Chemotherapy is usually not indicated in early and stable disease.[4] In addition, spontaneous regressions of the disease are exceptionally observed. However, patients with symptomatic and/or progressive disease should be immediately treated. In the routine clinical practice, therapy should not be initiated in patients who have asymptomatic CLL, including those with Rai stage 0 or Binet stage A until disease progression or unless disease-related symptoms are evident. There is no evidence that cytotoxic therapy based on alkylating agents has beneficial effects in patients with the indolent form of the disease.[5,6]

Chlorambucil with or without steroids has been the drug of choice for many years in patients with CLL.[7] This drug is still acceptable as the first line treatment of progressive CLL in frail, elderly patients with co morbidities, because of the apparent increase in toxicity of purine nucleoside analogs (PNAs) in this group of patients. The recent results of the German CLL study group (GCLLSG) support such a recommendation.[8] Bendamustine is a bifunctional alkylating agent composed of an alkylating nitrogen mustard group and a purine-like benzimidazole ring. Unlike other alkylating agents, bendamustine activates a base excision DNA repair pathway rather than an alkyl transferase repair mechanism. The safety and efficacy of bendamustine versus chlorambucil has been investigated in a randomised, open-label, comparative trial in previously untreated patients.[9] Overall response (OR) and complete response (CR) rates were significantly higher in patients treated with bendamustine. Moreover, the progression free survival (PFS) was longer after treatment with bendamustine (21.8 months) than after chlorambucil (8.0 months; $p < 0.0001$).

The purine nucleoside analogs (PNAs) – fludarabine, cladribine (2-CdA, 2-chlorodeoxyadenosine) and pentostatin also have been introduced for the treatment of CLL and fludarabine have become a standard drug for this leukaemia. Significantly higher OR, CR, and a longer progression free survival (PFS) in patients with CLL treated with fludarabine or 2-CdA were confirmed in randomised, multicenter trials.[10,11] However, the median survival time did not differ between patients treated with PNA and alkylating agents. Subsequently, the results of these randomized trials have shown that fludarabine combined with cyclophosphamide (FC) should be used instead of FA monotherapy in previously untreated patients with CLL.[12-14] In a randomised study that compared activity and toxicity of 2-CdA plus cyclophosphamide versus FC programme in previously untreated progressive or symptomatic CLL the efficacy and safety of both regimens were similar.[15]

Monoclonal antibodies, alemtuzumab and rituximab hold promise for further improvement. The results of recent clinical studies indicate that, rituximab in combination with a PNA or PNA and cyclophosphamide can increase the OR rate, CR rate and PFS. Recent treatment for younger, fit patients with CLL is combine cytotoxic chemotherapy with rituximab which has significantly improved the quality of response, duration of response, and survival in previously untreated and relapsed/refractory CLL. However, intensive treatment is often to toxic, particulary in older patients. The German CLL study group (GCLLSG) initiated a multicentre, multinational phase III trial, CLL8, to evaluate the efficacy and tolerability of rituximab combined with fludarabine and cyclophosphamide (R-FC) versus fludarabine and cyclophosphamide (FC) for the first-line treatment of patients with advanced CLL.[16] The OR rate was significantly higher in the R-FC arm (95%) compared to FC (88%) (p=0.001). The CR rate of the R-FC arm was 44% as compared to 27.0% in the FC arm ($p<0.0001$). At 3 years after randomisation, 65% of patients in the chemoimmunotherapy group were free of progression compared with 45% in the chemotherapy group ($p<0{\cdot}0001$); 87% were alive versus 83%, respectively (p=0·01). This randomized trial demonstrated for the first time that a specific first-line treatment for CLL improved OS. However, chemoimmunotherapy was more frequently associated with grade 3 and 4 neutropenia. R-FC is the first-line choice for younger, physically fit patients with CLL. Reducing the doses of fludarabine and cyclophosphamide while increasing the dose of rituximab demonstrated good efficacy combined with improved tolerability in previously untreated CLL patients.[17] R-FC regimen has also clinical activity in patients with refractory/relapsed disease. A randomized, multicentre, phase III study evaluated the efficacy and tolerability of R-FC versus FC regimens in relapsed or refractory patients with CLL (REACH study).[18] The PFS was significantly prolonged in the R FC arm (30.6 months) compared to FC (20.6 months, p =0.0002). An overall response rate was also higher for R-FC than for FC (70% *vs.* 58%, p=0.0034), due to superior CR rates (24% *vs.* 13%, p=0.0007). Recent clinical observations reveal that combination of rituximab with pentostatin or 2-CdA and cyclophosphamide (PCR or RCC regimens), are also highly active regimens in CLL.[19,20] When combined with cyclophosphamide, 2-CdA displays similar activity to FC in treatment-naive patients with advanced/progressive CLL in previously untreated CLL patients.[8] In addition, the results of high-dose methylprednisolone (HDMP) alone or in combination with rituximab in advanced CLL resistant to fludarabine have been recently reported by several groups.[21,22] These results demonstrate that HDMP in combination with rituximab is a useful treatment strategy in refractory CLL including patients with p53 abnormalities.

Alemtuzumab is a recombinant, humanised mAb which recognizes the CD52 antigen.[23] In previously untreated patients, an OR rate of more than 80% can be achieved. The results of the CAM 307 study may indicate that patients with 17p13.1 deletion, characterised by fluorescence *in situ* hybridisation (FISH) analysis, can be treated from the beginning with alemtuzumab.[24] In other CLL patients, this mAb should be reserved as second- or third-line treatment. However, alemtuzumab is ineffective in patients with bulky nodal disease (>5 cm) .

The next generations of anti-CD20 mAbs, potentially more effective than rituximab, have been generated in the recent years.[25] They have superior CDC and/or ADCC, increased binding to the low-affinity variants of the Fcγ receptor IIIa (FcγRIIIa, CD16) and reduced immunogenicity. Ofatumumab is a human, IgG1 mAb produced with a recombinant murine cell line (NS0) using standard mammalian cell cultivation and purification technologies. This antibody binds a membrane proximal epitope of CD20, composed of both the small- and large loop. Ofatumumab elicits more efficient CDC of target B-cells and releases only very slowly from the target compared with rituximab. Ofatumumab monotherapy is effective and well tolerated in patients with CLL, refractory to fludarabine and alemtuzumab.[26] Obinutuzumab (GA-101) is a humanised, glycoengineered, anti-

CD20 mAb, type II, anti-CD20, IgG1 mAb that differs significantly from other anti-CD20 mAbs.[27] Obinutuzumab is currently being evaluated in several ongoing phase II and phase III trials as a single agent and in combination with chemotherapy in NHL and CLL. The results of preclinical and clinical studies suggest that in patients with CLL, monoclonal antibodies with another target than CD20 can be useful in the treatment of this disease. These treatments include lumiliximab (anti-CD23), epratuzumab (anti-CD22), apolizumab (anti-MHC-II), galiximab (anti-CD80) and anti-CD40 monoclonal antibodies and TRU-016, small modular immunopharmaceutical (SMIP), a humanised fusion protein derived from key domains of an anti-CD37 antibody.

Other novel therapies are also being evaluated in CLL including immunomodulating agents, BCL-2 inhibitors, such as oblimersen, obatoclax, and ABT-263.[28] Immunomodulating agents are a new class of drugs that change expression of various cytokines and that co-stimulate immune effector cells. Lenalidomide is a second generation thalidomide analogue with possible immunomodulating and antiangiogenic properties, which may also modulate cytokine activity in the tumor microenvironment. Lenalidomide is orally available and has significant activity in CLL. The drug is currently under investigation in the treatment of CLL and shows encouraging results in the treatment of patients with high risk features such as del(17p)29. In addition, protein kinase inhibitors (KI), such as flavopiridol, spleen tyrosine kinase inhibitors (Fostamatinib disodium), Bruton's tyrosine kinase (PCI-32765, ibrutinib), and phosphatidylinositol 3-kinase inhibitors (CAL-101) are highly active and well tolerated in CLL patients irrespective of high risk genomic abnormalities and suggest that these drugs may be an important new targeted treatment approach for CLL30. In patients with CLL promising clinical results have been observed with a Bruton's tyrosine kinase (Btk) inhibitor - ibrutinib (PCI-32765), a spleen tyrosine kinase (Syk) inhibitor-fostamatinib disodium (FosD) and a selective inhibitor of PI3K - CAL-101(GS-1101.[30] KIs are available in oral preparations and are given as continuous treatment. These drugs seem to be active in traditionally poor risk disease groups, including fludarabine refractory patients and patients with bulky lymphadenopathy. These agents induce rapid resolution of lymphadenoapathy and a transient increase of lymphocytosis due to mobilization of CLL cells into the peripheral blood. However, after several months of continuous therapy, response can be achieved in a substantial number of patients.

In conclusion, alkylating agents still have an important place in the routine management of older, unfit CLL patients. Purine nucleoside analogs, particularly combined with cyclophosphamide and rituximab, should be routinely used as first line therapy in younger patients. Monoclonal antibodies and kinase inhibitors will, probably, present the foundation for innovative therapeutic strategies in this leukaemia. This is an exciting time for the development of new, effective drugs for the treatment of CLL, which should significantly improve the prognosis of this disease in the near future.

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The evolving role of stem cell transplantation in chronic lymphocytic leukemia

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The purpose of this presentation is to describe the current role and perspectives of allogeneic stem cell transplantation (alloSCT) in the treatment of chronic lymphocytic leukaemia (CLL). In particular, it will be outlined that

1. Clinical trials and minimal residual disease (MRD) studies provide clear evidence that graft-versus-leukaemia activity (GVL) is working in CLL and can provide long-term MRD-free survival in up to 50% of patients undergoing alloSCT.
2. AlloSCT is effective also in poor-risk CLL as defined by the EBMT transplant consensus (purine analog refractoriness, early relapse (<2 years) after intensive therapy, TP53 gene abnormalities).
3. Novel forms of (reduced intensity) conditioning (RIC) has resulted in dramatic reduction of early morbidity and mortality of alloSCT in CLL, making this procedure now suitable for comorbid and elderly patients.
4. Long-term NRM after RIC alloSCT is still ranging from 15% to 25%, and long-term morbidity and quality of life is largely determined by chronic GVHD (which is also the most important prerequisite for CLL eradication).
5. Indications for alloSCT in CLL are those defined in the EBMT CLL Transplant Consensus, but also patient-related risk factors, such as age, comorbidity, and actual disease activity, have to be considered when the decision about alloSCT is made.
6. In eligible patients alloSCT should be considered as soon as one of the EBMT criteria is met.
7. Preliminary evidence suggests that alloSCT indeed can change the natural history of poor-risk CLL, and novel CLL-targeting drugs may have the potential to further improve transplant outcome.

Abstracts oral presentations O.1 - O.8

O.1 Exome sequencing identifies mutation of the ribosome in T-ALL

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Background

T-cell acute lymphoblastic leukemia (T-ALL) is caused by accumulation of mutations in developing T-cells. Major driver mutations in T-ALL include hyperactivity of the NOTCH1 pathway, activation of JAK/STAT signaling, overexpression of a set of transcription factors, and loss of cell cycle control. Although pediatric T-ALL patients respond well to the current chemotherapy regiments, the treatment is toxic and the survival of older patients is still below 40%.

Aims and methods

To gain insight in the mutation spectrum in T-ALL and to identify potential new targets for therapy, we performed exome sequencing on 39 paired diagnosis-remission T-ALL samples, 28 diagnosis only samples and 17 T-ALL cell lines. Our patient cohort represented all different molecular subgroups in T-ALL and contained adult and pediatric samples.

Results

We initially limited our analysis to the 39 paired sample sets for discovery of novel somatic mutations. We detected protein-altering mutations in 508 genes, with an average of 8.2 mutations in pediatric and 21.0 in adult T-ALL. Based on stringent filtering, we predict 15 of these genes to be oncogenic drivers, including seven genes that were not previously implicated in T-ALL (*CNOT3, RPL10, RPL5, ODZ2, TET1, KDM6A* and *MAGEC3*). Interestingly, two of these novel genes (RPL10 and RPL5) encoded proteins that are part of the ribosome, the molecular machinery in the cell that translates mRNA into proteins. Complementary Sanger sequencing of RPL5 and RPL10 in an independent T-ALL cohort confirmed that 12/122 (9.8%) pediatric and 3/89 (3.4%) adult T-ALLs harbored mutations in *RPL10* or *RPL5*. Yeast and lymphoid cells expressing the T-ALL associated RPL10 mutants showed a clear defect in the generation of mature ribosomes.

Summary and conclusions

Our data provide insights in the landscape of mutations in T-ALL and show that adults contain 2.5 times more mutations than children. We identify mutation of the ribosome as a novel oncogenic factor and potential target for therapy in T-ALL.

O.2 Nanobodies as new imaging tools in multiple myeloma: a study in the 5T2MM murine model.

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Nanobodies are single-domain, antigen-binding fragments derived from the heavy chain-only antibodies naturally occurring in Camelidae. Here we studied the use of anti-idiotype Nanobodies as an imaging tool in Multiple Myeloma (MM) using the 5T2MM murine model. This model is highly analogous to the human disease and is characterised by tumour expansion in the bone marrow, production and secretion of monoclonal paraprotein and bone lesions. After dromedary immunisation with 5T2MM idiotype we were able to isolate anti-idiotype Nanobodies. Their high affinity and specificity was demonstrated by ELISA, affinity binding assay and FACS analysis. Based on these results we selected the best binder (R3B23) for further *in vivo* analysis.

R3B23 was labeled with ^{99m}Tc-tricarbonyl (^{99m}Tc-R3B23) for *in vivo* tracing. Healthy C57Bl/KaLwRij mice or 5T2MM diseased mice were i.v. injected with ^{99m}Tc-R3B23. One hour post injection the mice were sacrificed and the radioactivity present in different organs and tissues was measured. In the naive mice we observed high kidney and bladder uptake, and very low uptake in the other organs and tissues, indicating fast renal clearance. In the diseased animals, increased radioactivity was detected in all the examined organs including blood, liver, spleen and bone. Using ^{99m}Tc-R3B23 we monitored disease progression: we scanned the 5T2MM mice by pinhole SPECT and micro-CT scan at different time points starting at week 1 after inoculation with 5T2MM cells into nave mice. Image analysis showed an increased ^{99m}Tc-R3B23 uptake starting at week 7 after tumor inoculation. At this time point no circulating M-spike could be detected by capillary electrophoresis.

^{99m}Tc-R3B23 was able to monitor therapeutic response in 5T2MM mice. After three weeks treatment with velcade (two days per week) we saw a clear decrease in Nanobody uptake compared to the untreated group.

Our study shows that anti-idiotype Nanobodies are a new, sensitive, specific and non-invasive imaging tool in MM and provides the rationale for further studies on their clinical application. In addition Nanobodies can be coupled to toxic agents to target the MM cells.

O.3 Donor Characteristics As Pretransplant Predictive Factors of Long-Term Outcomes After Allogeneic Peripheral Blood (PB) Stem Cell Transplantation (SCT) From HLA-Matched Related and Unrelated Donors in Patients with Hematologic Malignancies

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Optimal strategy for donor selection is still controversial. We evaluated the impact of donor type (10/10 HLA-matched unrelated (MUD) versus matched related (MRD)) and other donor traits on long term outcomes of patients with hematologic malignancies after PB SCT.

Methods

We analyzed 442 consecutive patients with hematologic malignancies who were transplanted with PB either from MUD (n= 164) or MRD (n=278) from 01/2000 to 12/2010 at Saint-Louis Hospital (Paris). Reduced intensity conditioning was performed in 2/3 of patients. We assessed impact of donor type, age, gender, CMV and ABO status on outcomes. MUD and MRD SCT were balanced for patient age, disease risk and conditioning. Median donor age was fourty years. As the upper age limit for voluntary PB donation was 60y, we completed our analysis by performing three groups according to donor type and age (MUD, MRD<60y and MRD=60y). Thirty-six patients were transplanted with MRD=60y.

Results
Median FU was 36 months and 25% of patients had a FU of at least 60 months. The 5y-CIf of cGVHD was 58%. Sex mismatch (F>M) increased risk of cGVHD (HR: 1.41, *P*=.02). The 5y-CIf of relapse was 34% and was higher with MRD than MUD (39% *vs.* 24%, *P*=.038). Only MRD=60y resulted in significant higher risk of relapse than MUD (HR 2.46, *P*=.006) while MRD <60y had similar risk. The 5y-NRM was 26%. MUD *vs.* MRD was associated with higher NRM (HR: 1.84, *P*=.005). The 5y-OS was 46% and was similar with MUD and MRD. MRD=60y appeared to have notable low 5y-OS (6%, 6%). Transplantation from MRD=60y was associated with higher risk of late (=18 months) mortality (HR: 4.36, *P*=.007) than MUD (Fig. 1).

Conclusion
After PB SCT, MUD provided higher NRM but better disease control and similar OS than MRD. A sex mismatched donor (F>M) was associated with higher risk of cGVHD. We observed notable poor outcome for patients transplanted with MRD=60y. One may thus question HCT with old MRD when a younger MUD is available.

O.4 Combination of regulatory T cells injection with rapamycin for treatment of chronic Graft-versus-Host disease

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Background
Chronic graft-versus-host disease (cGvHD) occurs up to 50 % in long-term survivors and is one of the main complications after allo-HSCT. Donor $CD4^+$ and regulatory T cells (Tregs) are the key-players in its pathogenesis. Moreover, rapamycin, a mTor inhibitor, could suppress activation and proliferation of effector T cells and expand *in vitro* Tregs.

Aims
To assess the combined treatment of Tregs and rapamycin injections *in vivo* for cGvHD.

Results
Lethally irradiated Balb/C mice were injected with $10x10^6$ bone marrow cells and $70x10^6$ splenocytes from B10.D2 donor mice. Twenty-one days later, the treatments were started (PBS, rapamycin 1 mg/kg/Day, Tregs 1.10^6 cells or rapamycin 1 mg/kg/Day + Tregs 1.10^6 cells). No significant differences were observed between survival of PBS-treated (Median: 40 days) compared to rapamycin alone or Tregs alone (Median: 46 days, *p*=0.1390; Median: 46 days, *p*=0.2450 respectively) while survival of mice receiving rapamycin and Tregs was increased (*p*=0.0074). Twenty-one days after starting the treatment, number of $CD4^+$ T cells was significantly decreased in Tregs (37.0010.00; *p*=0.0303) and Tregs + rapamycin-treated (27.0056.00; *p*=0.0293) mice compared to PBS mice (95.00100.50). Proliferation of $CD4^+$ T cells (assessed by flow cytometry unsing Ki67) was only significantly decreased in Tregs + rapamycin-treated mice (19.609.17 versus 36.8012.40; *p*=0.0043). Number of cells per micorliters and proliferation of both effector and central memory $CD4^+$ T cells were significantly decreased in Tregs + rapamycin-treated mice compared to PBS mice. Number of $CD8^+$ T cells was significantly decreased in rapamycin (56.0063.00; *p*=0.0082) and Tregs + rapamycin-treated (58.5077.80; *p*=0.0082) mice compared to PBS mice (144.00103.80). Despite a significant increase in the percentage of Tregs in rapamycin 1 mg/kg/Day (20.3043.15; *p*=0.0519), Tregs 1.106 cells (95.6559.75; *p*=0.0190) or rapamycin 1 mg/kg/Day + Tregs 1.106 cells groups (29.6066.30; *p*=0.0043) compared to PBS-treated mice (15.353.80), no significant differences were seen in the number per micorliter.

Conclusion
Regulatory T cells injection combined with rapamycin daily administration seems to treat cGvHD *in vivo* by combining the beneficial effect of these treatments.

O.5 In vitro generation of antigen-specific T-cells from hematopoietic progenitor cells: a new and promising immunotherapeutic strategy

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Introduction
Transfer of high-affinity tumor-specific T-cell receptor (TCR) genes into polyclonal peripheral blood T-cells is an attractive immunotherapeutic strategy against malignancies and viruses. However, inappropriate crosspairing between introduced and endogenous TCR chains can result in suboptimal activity and unpredicted, potentially harmful antigen-specificities. Efficient *in vitro* generation of antigen-specific T-cells from $CD34^+$ hematopoietic progenitor cells (HPCs) may eliminate these restrictions, based on the hypothesis that early introduction of rearranged TCRa and TCR chains might result in allelic exclusion of the endogenous TCRa and/or TCR locus. We and others have previously shown that HPCs commit to the T-cell lineage and become $CD4^+CD8^+$ double positive (DP) precursors when cultured on OP9-DL1 stromal cells.

Results
$CD34^+$ HPCs from human postnatal thymus were retrovirally transduced to express the TCRa and TCR chains of HLA-A2 restricted TCRs recognising epitopes of cytomegalovirus (CMV pp65) or Wilms' tumour 1 (WT1). Differentiation in transduced cultures was studied. We confirmed earlier reports showing that terminal maturation of TCR-transduced DP cells to mature CD8 single positive (SP) cells occurs, albeit at low efficiency. We hypothesised that the observed maturation involved selection by TCR binding to HLA class I /peptide complexes present in culture. Therefore, we added the respective agonist peptide to the cultures. This induced rapid phenotypical maturation to $CD27^+CD1^-$ of the majority of $TCRa^+$ DP cells. Antigen presentation by $HLA\text{-}A2^+$ dendritic cells, HLA-A2+ tumour cell lines, and even cross-presentation by $HLA\text{-}A2^+$ T-cell progenitors, but not by $HLA\text{-}A2^-$ cells, induced this maturation process. The mature cells are $CD8a^+$ or $CD8aa^+$SP and $CD4^-CD8^-$ cells. These T-cells expanded upon culture with PHA and IL-2 on irradiated feeders, indicating functionality. Upon activation, specific killing of T2 cells loaded with agonist peptide, was observed. *In vitro* generated T-cells showed clearly higher percentages of tetramer-positive cells compared with TCR-transduced peripheral blood T-cells. Spectratyping revealed major inhibition of endogenous TCRa and TCR gene rearrangements.

Conclusion
In vitro generation of functional antigen-specific T-cells from $CD34^+$ HPCs is a promising new immunotherapeutic strategy.

O.6 Infusion of CliniMACS® (Miltenyi Biotec) enriched regulatory T-cells delays experimental xenogeneic graft-versus-host disease.

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Background
Graft-Versus-Host-Disease (GVHD) is a life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT). Animal models have demonstrated that Treg infusion could prevent otherwise lethal GVHD in mice given grafts from MHC-disparate donors. Here, we assessed the ability of clinical-grade isolated human Treg to attenuate experimental xenogeneic GVHD.

Material and methods
Human Treg were isolated from cytapheresis products with the Miltenyi CliniMacs system using a two steps procedure (CD8 and CD19 depletion followed by CD25 positive selection) in six independent experiments with six different healthy volunteer donors. Sub-lethally (2.5 Gy) irradiated NSG mice were given $2x10^6$ cytapheresis product cells i.v. without (PBMC group) or with $1x10^6$ Tregs (PBMC+Treg group), while other NSG mice received only $2x10^6$ Treg (also in i.v.; Treg group). Mice in terminal stage GVHD were euthanised.

Results
After the selection, we obtained a CD25 enriched fraction including a median of 1.81 x108 cells and containing 59 +/- 6% or 66 +/- 6% Treg defined as either $CD45^+CD4^+CD25^{High}FoxP3^+$ cells or $CD45^+CD4^+CD25^{high}CD127^{low}$ cells. In all experiments but the last (a technical problem dramatically impacts the efficiency of this selection), Treg co-transfusion significantly delayed death from xenogeneic GVHD. Specifically, median survivals in PBMC versus PBMC+Treg mice were 30 *vs* 56 days (p=0.015), 123.5 *vs* >162 days (p=0.23), 25.5 *vs* 70 days (p=0.012), 13 *vs* 16 days (p=0.038), 27 *vs* 49 days (p=0.061), and 46 *vs* 47 days (p=0.338) respectively. Further, none of the mice given only Treg experienced signs of GVHD, while, interestingly, the $CD4^+$ cells found in these mice 27 days after transplantation were mainly conventional T cells ($CD25^+FoxP3^+$ cells in human $CD4^+$ total cells were only 2.1%, 3.1% and 17.7% in spleen, bone marrow and blood, respectively while 80.2% were grafted).

Conclusion
Treg infusion delayed the occurrence of xenogeneic GVHD without showing any toxicity in this murine model.

O.7 Erythropoietin therapy after allogeneic hematopoietic cell transplantation : a prospective randomised trial

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Based on the impairment of erythropoietin production after allogeneic hematopoietic cell transplantation (HCT), we previously reported in a phase-2 trial that recombinant human erythropoietin (rhEPO) therapy was very efficient when started one month after transplantation. We also demonstrated that anemia after non-myeloabalative (NM) HCT was less sensitive to rhEPO therapy than after conventional allogeneic HCT. This prompted us to confirm these findings in a prospective randomised trial.
One hundred and thirty-one patients were randomised (1:1) between no treatment (arm 1) or erythropoietin (Neorecormon) at the dose of 500 U/kg/week (arm 2). Once the target Hb (13g/dL) has been attained, the dose of rhEPO was reduced by half, while it was withheld when Hb was = 14g/dL. Cohort A included 42 patients on day 28 after myeloablative HCT, cohort B 39 patients on day 28 after NMHCT, and cohort C 50 patients on day 0 of NMHCT. Primary endpoints included proportion of complete correctors (i.e. patients reaching Hb = 13g/dL) and median time to achieve Hb correction in each arm.
The proportion of complete correctors before day 126 post-transplant was 0% in group 1A *vs* 52.4% in group 2A, 0% in group 1B *vs* 69.5% in group 2B and 19.1% in group 1C *vs* 70.2% in group 2C. Median time to achieve Hb = 13g/dL was not reached in group 1B *vs* 49 days in group 2B; 363 and 59 days in groups 1A and 1B respectively and 363 and 87 days in groups 3A and 3B respectively (figure 1). Hb evolution in each group is shown in figure 2. Seventy-one patients (47/62 in control groups and 24/57 in treated groups, p=0.0003) required red blood cell transfusions. The difference was most pronounced in cohort B. There was no difference in rates of thrombo-embolic events or other complications between the two arms. In conclusion, this is the first trial to demonstrate that EPO therapy hastens erythroid recovery and decreases transfusion requirements when started one month after allogeneic HCT.

O.8 The value of asparaginase intensification for children with low and average risk acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL) in the EORTC-CLG Randomized Phase III Trial 58951

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Background
Asparaginase (ASP) is an essential component in combination chemotherapy for childhood ALL. However, the optimal number of ASP-administrations is still unknown. We conducted a randomised phase III trial comparing conventional *E.coli*-ASP-regimen (short-ASP, 12 doses) with prolonged *E.coli*-ASP-therapy (long-ASP, 24 doses).

Methods
The EORTC-CLG 58951 trial was open to de novo ALL or NHL patients (pts) <18y. This study addressed two main randomised questions. The first evaluated the value of dexamethasone (DEX, 6mg/m/d) *vs* prednisolone (PRED, 60mg/m/d) in induction for all patients. In the second question all non-VHR pts were randomised for either short- or long-ASP. All patients received 8x10000U/m in induction. In the short-ASP-arm patients received 4x10000U/m in reinduction; patients in the long-ASP-arm received 8x5000U/m *E. coli*-ASP-injections in consolidation and eight (4x10000U/m + 4x 5000U/m) in reinduction. Patients with grade =2 allergy to *E.coli*-ASP were switched to equivalent doses of *Erwinia* or PEG-ASP.

Results
Between 12/1998 and 08/2008, 1552 patients were randomly assigned to receive long-ASP (n=775) or short-ASP (n=777). The 8-year DFS rate was 87.0% in the long-ASP and 84.2% in short-ASP-group (hazard ratio (HR) = 0.87, 95% CI 0.66-1.14, 2-sided logrank p=0.30). The 8-year OS rate was comparable in both treatment arms: 92.6% in the long-ASP-group and 91.3% in the short-ASP-group (HR = 0.89, 95% CI 0.61-1.29, p=0.53). Similar treatment differences were observed in each risk group, in PRED *vs* DEX arm, and B- and T-lineage ALL-patients. The incidence of grade 3-4 infection was higher in the long- versus short-ASP-group during consolidation (25.2% *vs* 14.5%) and re-induction (22.6% *vs* 15.9%). This difference was more pronounced in patients who received DEX in induction (27.3% *vs* 11.6%). During the whole treatment period, the incidence of grade 2-4 allergy was 32.8% in the long-ASP-arm and 21.8% in the short-ASP-arm.

Conclusion
At long follow-up prolonged *E.coli* asparaginase therapy in conso-

lidation and reinduction for VLR and AR patients did not improve significantly the outcome. Intensive ASP-treatment did increase infection rate and resulted in more grade 2-4 allergy.

Abstract posters lymphoid malignancies (P.01-P.25)

P.01 NUP214-ABL1 mediated cell proliferation in T-ALL depends on the LCK kinase and various interacting proteins

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Background
The NUP214-ABL1 fusion protein is a constitutively active protein tyrosine kinase that is found in 6% of T-cell leukaemia (T-ALL) patients and that stimulates proliferation and survival of the leukaemia cells. Although NUP214-ABL1 is sensitive to ABL1 kinase inhibitors, evidence is accumulating that development of resistance to these compounds may represent a major clinical problem.

Aim
We aimed at identifying additional drug targets in the largely unknown NUP214-ABL1 signaling network that could eventually be used to overcome resistance in NUP214-ABL1 positive T-ALL.

Results
Because of the importance of SRC family kinases for the related BCR-ABL1 fusion kinase, we first explored the role of SRC kinases in survival of NUP214-ABL1 positive cells. We identify and validate the SRC family kinase LCK as a critical protein for the proliferation and survival of T-cells expressing NUP214-ABL1. These findings underscore the potential of the ABL/SRC kinase inhibitors dasatinib and bosutinib for the treatment of NUP214-ABL1 positive T-ALL.In addition, we used mass spectrometry to identify protein interaction partners of NUP214-ABL1. Our results indicate that the signaling network of NUP214-ABL1 is distinct from that previously reported for BCR-ABL1. Using an siRNA screen of identified NUP214-ABL1 interaction partners, we found that the MAD2L1, NUP155, and SMC4 proteins are strictly required for the proliferation of NUP214-ABL1 positive T-ALL cells.

Conclusion
Our results identify LCK, MAD2L1, NUP155 and SMC4 as new potential drug targets in NUP214-ABL1 positive T-ALL and suggest that dual ABL1/SRC inhibitors may be superior to ABL1 inhibitors for NUP214-ABL1 positive patients.

P.02 H3K27me3 erasers, novel targets in T-cell acute lymphoblastic leukaemia

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T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive malignancy of thymocytes affecting children, adolescents and adults. T-ALL is characterised by a differentiation arrest at specific stages of T-cell development, primarily due to deregulated expression of T-cell specific genes. Recently, our group identified five frameshift mutations in the *Ubiquitously transcribed tetratricopeptide repeat (UTX)* gene, located on chromosome X, in 35 primary T-ALL patient samples (14.3%). T-ALL is characterized by an unequal gender distribution (male/female ratio: ~3/1). Interestingly, *UTX* mutations were detected exclusively in male T-ALL patient samples. Furthermore, the *Jumonji domain-containing protein 3 (JMJD3)*, another member of the *UTX* family, harbored missense and indel mutations in 2/35 (5.7%) primary T-ALL samples. In addition, we identified a focal deletion in the *UTY* gene, a paralogue of *UTX* located at the Y-chromosome, in 1/5 *UTX* mutant patient samples. The UTX and JMJD3 genes function as histone demethylases. More specifically, they act by demethylating di- and trimethyl groups on lysine 27 on the tail of histone 3 (H3K27me2/3; silencing marker) thereby activating its target genes. The counterpart of the H3K27me3 erasers, namely the *polycomb repressive complex 2 (PRC2)* with main members *EZH2, SUZ12* and *EED*, was recently identified as a mutational target in T-ALL. In our T-ALL cohort, we identified two *SUZ12* deletions and one *SUZ12* mutation as well as a deletion encompassing *EED*. Surprisingly, we didn't find any genetic defects of *EZH2* in our patient cohort. To further delineate the role of histone modifier defects in T-ALL, we performed loss-of-function studies in *in vitro* model systems and *in vivo* using a *NOTCH1*-induced T-ALL mouse model. Loss of *UTX, UTY, SUZ12* and *EZH2* resulted in a strong acceleration in leukaemia onset in the *NOTCH1* mouse model. To further elucidate the molecular changes that result from the depletion of these chromatin modifiers, we are generating gene expression profiles and ChIP-sequencing maps of histone marks. Finally, we are also exploring the therapeutic effects of *EZH2* inhibition in the *UTX* driven tumours.

P.03 Extending the functional redundant cooperative miRNA tumour suppressor and oncogene network in T-ALL

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Introduction
T-cell acute lymphoblastic leukaemia (T-ALL) is a hematological malignancy arising through cooperation between genetic defects affecting proliferation, survival, cell cycle and T-cell differentiation. We recently identified miRNAs implicated in a cooperative oncoMir - tumour suppressor gene network in T-ALL. These miRNAs are functionally redundant in normal cells and collectively upregulated in T-ALL cells resulting in downregulation of tumour suppressor gene expression.

Methods
In order to further expand this regulatory miRNA network, we performed a miRNAome wide screen for 3'UTRs of five important tumour suppressor and oncogenes implicated in T-ALL, including PHF6 and NOTCH1. Using this approach, we could detect all miRNAs targeting the 3'UTR of a given suppressor or oncogene using a library of 470 miRNA mimic molecules.

Results
Analysis of repeated screens underscored the high reproducibility of the assay and top hits included previously reported *bona fide* miRNAs for T-ALL. In addition to the predicted hits also non-predicted miRNAs were identified, thus drastically increasing the

number of relevant miRNAs targeting the genes of interest. Following correlation analysis of the corresponding target gene and miRNA expression levels of 64 primary T-ALL samples, 20 T-ALL cell lines and normal T-cell subsets of human donors representing various stages of normal T-cell development, integrative mRNA - miRNA expression analyses *(http://www.mirnabodymap.org)* revealed putative miRNA functions.
In addition to the network analysis, we particularly focused on miRNAs targeting NOTCH1 and PHF6 for which currently further *in vitro* and *in vivo* assays are ongoing to confirm their role in normal T-cell development and T-ALL oncogenesis.

Conclusions
Our study provides a highly novel systems level assessment of the collectively perturbed miRNAs controlling a cooperative network of tumour suppressor and oncogenes implicated in T-ALL oncogenesis. The detailed dissection of these networks offers potential for development of miRNA based therapeutic strategies and molecular monitoring of therapy response and resistance.

P.04 HDAC in Chronic Lymphocytic Leukaemia: expression profile, enzymatic activity and prognostic significance

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Background
Histone deacetylases (HDAC) play a crucial role in chromatin structure and gene expression. Their deregulation has been reported in various cancers.

Design and methods
We performed a complete and comprehensive study of the 18 HDACs (including Sirtuin; SIRT) expression by real-time PCR in a cohort of 200 chronic lymphocytic leukaemia (CLL) patients with a median follow-up of 77 months and compared it with normal B cells. These data were after correlated with patient evolution. Next, we assessed the deacetylation power of CLL protein extracts (n=45).

Results
We showed significant deregulation (mostly up-regulation) of HDACs in CLL compared to normal controls. In terms of clinical significance, HDAC6 was correlated with treatment-free survival (TFS); HDAC3, SIRT2, 3 and 6 were correlated with overall survival (OS). A multivariate Cox regression stepwise analysis indicated that HDAC6, 7, 10 and SIRT3 were TFS independent predictors. Interestingly, poor prognosis was associated with an overexpression of HDAC7 and 10 but an underexpression of HDAC6 and SIRT3. Therefore, these factors were combined in a TFS score (1 point for a bad prognosis occurrence): patients with a score of 0-1-2, 3 and 4 had a median TFS of 107, 57 and 26 months, respectively (HR=4.03, P<0.0001). For OS, SIRT5 and 6 allowed stratification into 3 groups, with a median OS of >360, 237 and 94 months (HR=6.38, P<0.0001). On the other hand, we observed that deacetylation power of CLL protein lysates is lower (5.3 0.2 pmol of deacetylated substrate) than control group (7.6 0.5 pmol; *P*= 0.0013). Interestingly, patients with high deacetylation activity displayed a shorter OS (151.37 months) than those with low deacetylation power (OS>360 months; *P*= 0.0158).

Conclusion
These results highlight the complex impact of HDAC expression on CLL clinical course. Moreover, we show for the first time that high HDAC enzymatic activity is associated with a poor prognosis in CLL encouraging the use of HDAC inhibitors in CLL treatment.

P.05 The Combination of Decitabine and the Histone Deacetylase Inhibitor JNJ-26481585 Has Synergistic Anti-Myeloma Activity

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Epigenetic modulating agents, including DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi), are of interest for their anti-tumour properties. Decitabine (DAC) is a FDA approved DNMTi for treatment of myelodysplastic syndrome, while JNJ-26481585 (JNJ-585) is a HDACi with potent anti-myeloma activity. Several studies revealed that DNMTi and HDACi exert their anti-tumour response by reversing epigenetic changes resulting in alterations in the transcriptome. In addition, DNMTi and HDACi exert direct cytotoxic effects. Limited studies are available on DAC alone and in combination with HDACi in multiple myeloma. Therefore we assessed the potential synergistic effect of DAC and JNJ-585 in MM.
For *in vitro* experiments, we focused on human (OPM-2, RPMI 8226) and murine (5T33MM) cell lines. Using Cell-Titer-Glo, DAC and JNJ-585 showed synergistic anti-myeloma effects. This was confirmed by an AnnexinV-7AAD apoptosis assay. In general, apoptosis was associated with H2AX phosphorylation, caspase- and PARP-cleavage, which was more pronounced in the combination. In the OPM-2 cells, we also observed Mcl-1 cleavage and Bim upregulation. DAC and JNJ-585 also affected cell cycle progression. In the 5T33MMvt cells, DAC induced a G2/M-phase arrest while JNJ-585 induced a G0/G1-phase arrest. Combining both agents cancelled out the observed arrests and resulted in subG1 increase. In OPM-2 cells, DAC did not induce an arrest while JNJ-585 induced a G0/G1-phase arrest, which was maintained in the combination. In support of the cell cycle arrest, p21 and p27 were upregulated in both cell lines. Lastly, in the 5T33MM murine model, combination treatment resulted in significant lower serum M-spike, bone marrow tumour load and increased survival probability compared to single agent treatment. In conclusion, we demonstrated a synergistic anti-myeloma effect of DAC and JNJ-585. This was associated with a DNA damage response, cell cycle arrest, caspase activation and disruption of Bcl-2 family balance. DAC and JNJ-585 treatment *in vivo* resulted in slower disease progression suggesting clinical relevance.

P.06 Safety and efficacy of abbreviated induction with oral fludarabine (F) and cyclophosphamide (C) combined with dose-dense iv rituximab (R) in previously untreated patients with chronic lymphocytic leukaemia (CLL) aged > 65 years: results of a multicentre trial (LLC 2007 SA) on behalf of the French

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Elderly population is underrepresented in CLL trials and it is still unclear how FCR should be applied in this subgroup. We report

safety/efficacy of abbreviated FC and dose-dense R in untreated CLL patients > 65 y without del17p enrolled in the French intergroup LLC 2007 SA trial. Stage B/C fit (CIRS = 6, CCR > 60 ml/min, PS = 1) patients (n=194, median age 71 y, 65% males, unmutated *IgVH* 56%, del11q 18%, t12 15%, del13q 57%) were included. FCR consisted of four monthly cycles of oral FC (F 40 mg/msq/d and C 250 mg/msq/d, x 3d) and iv R (375 mg/msq d1 cycle 1, 500 mg/msq d14 cycle 1, d1 and 14 of cycle 2, and d1 of cycles 3 and 4). 86% received all four cycles of FCR. Less than 5% of patients could not receive d14 rituximab. Dose delay and dose reduction for cycles 2, 3 and 4 were 12% and 7%, 14% and 8%, 15% and 11%, respectively. Neutropenia g3/4 occurred after cycle 1, 2, 3 and 4, in 46%, 50%, 53%, and 46% of the patients. G-CSF was given in 32%, 46%, 48%, and 52% of them after cycles 1, 2, 3, and 4. G3/4 infectious events occurred in 6.2%, 4.8%, 7.6%, and 6.2% of the patients after each of the 4 cycles. In total, 6.3% of the 732 cycles were followed by febrile neutropenia or infection qualified as SAE. Six deaths (all infections) occurred during induction (3.1% death rate from immediate toxicity). CR was observed in 19.7%, nPR in 2.7%, and PR in 73.9%, for an ORR of 96.3%. According to the updated guidelines of 2008 (Hallek), CR, CRi, and PR rates were 19.7%, 13.3%, and 63.3%, respectively. In conclusion, this approach could enable the safe administration of FCR to elderly fit CLL patients in first line. The response rate is high, and further analysis of MRD eradication is ongoing and will be presented. Long-term toxicity will be scrutinised.

P.07 Interim analysis of the randomized EORTC/LYSA/FIL Intergroup H10 trial on early PET-scan driven treatment adaptation in stage I/II Hodgkin lymphoma

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Introduction

Early stage classical Hodgkin lymphoma (cHL) is highly curable with a combination of chemotherapy and radiotherapy (RT). FDG-PET (PET) has emerged as a potential new tool to early select good prognosis patients after 2 ABVD. In the current trial, early PET is used to guide therapy.

Patients and methods

All patients with a stage I and II supra-diaphragmatic cHL, between 15 and 70 years old are eligible for the study and stratified according to LYSA/EORTC criteria in 2 groups: Favorable (F) and unfavorable (U). The standard treatment is ABVDx3 (F) and ABVDx4 (U) and 30Gy involved-node RT (IN-RT). The experimental arm consisted of ABVDx2 followed by a PET: if the PET is negative, patients receive 2 additional cycles of ABVD (F) or 4 ABVD (U) and no RT. If the PET is positive, patients received BEACOPPesc x2 and 30 Gy IN-RT.

Results

In the F PET negative group, 188 patients were included in the standard arm, and 193 in the experimental arm. A total of ten events occurred: one in the standard arm and nine in the experimental arm. Futility was declared (p-value=0.017<0.102). The estimated hazard ratio was 9.36 (79.6% CI=[2.45-35.73]). In the U early PET negative group, 251 patients were included in the standard arm and 268 in the experimental arm. A total of twenty-three events occurred in this group: seven in the standard arm and sixteen in the experimental arm. Futility was declared (p-value=0.026<0.098). The estimated hazard ratio was 2.42 (80.4% CI=[1.35-4.36]). The IA for the early PET positive group gave no reason for stopping this part of the trial. Final accrual was reached in June 2011 with 1950 included patients.

Conclusion

The planned futility IA of the H10 trial shows that the risk of early relapse in non-irradiated patients with stage I-II cHL was significantly higher than in standard arm.

P.08 Comparison of Long-Term Outcome between Belgian and Vietnamese Children Treated for Acute Lymphoblastic Leukaemia according to Fralle 2000 protocol

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Background

Acute lymphoblastic leukaemia (ALL) is the most common type of childhood cancer in East Asian, Caucasians and in the United States. Previous studies have shown poorer survival from childhood ALL among Asian compared to Caucasian populations. The primary goal of this study was to compare the outcome of Belgian and Vietnamese children with ALL, treated with the Fralle 2000 protocol (French acute lymphoblastic leukaemia).

Patients and methods

The Belgian series included 107 patients followed at Cliniques universitaires Saint-Luc (UCL), Brussels, Belgium between 2001 and 2011. The Vietnamese series included 166 patients from Blood Transfusion and Hematology Hospital, University of Medicine Pham Ngoc Thach (UPNT) at Ho Chi Minh city, Vietnam and followed between 2005 and 2011. Relative risk of Adverse Drug Reactions (ADR) was computed between Belgian and Vietnamese children using a Log-Binomial Regression model. Hazard ratios (HR) were computed for the overall survival and relapse free survival using a cox proportional hazard model.

Results

Data from both groups were comparable in terms of age at diagnosis, sex ratio, initial white blood cell count, cytogenetic, and steroid responsiveness at day 8. A higher prevalence of L2 type-ALL according to the FAB classification was found in Vietnamese children (86.5% *vs* 44.9% in Belgium). Vietnamese children had a comparable risk of ADR (adjusted RR =1.02, p-value=0.89) but a higher relative risk of relapse (adjusted HR = 3.79, p<0.01), and a lower overall survival rate (adjusted HR = 4.03, p<0.01). The number of deaths caused by toxicity was comparable in both groups but Vietnamese children had a higher risk of death caused by relapse (Figure 1).

Conclusions

Racial differences in pharmacogenetics of drugs as well as additional factors such as social status, lack of antibiotic prophylaxis or delayed

access to care due to remoteness may explain the poorer overall survival observed in ALL Vietnamese children.

Appendix: legend for Figure

P.09 Exome sequencing identifies mutation of CNOT3 in T-cell acute lymphoblastic leukaemia

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T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive neoplastic disorder that is caused by the cooperation of multiple oncogenic lesions. Despite major improvements in our understanding of the molecular genetics of T-ALL, the mechanisms that lead to the abnormal proliferation and/or survival of T-lymphoblasts remain largely unknown.
In order to further increase our understanding of the genetic defects present in T-ALL, we have recently performed whole exome sequencing on a subset of 67 T-ALL cases. Using this approach, we detected fifteen oncogenic drivers, including seven genes that were not previously implicated in T-ALL. Our candidate driver list contained the *CNOT3* gene, with four patient samples carrying somatic mutations (5.9%; 4/67). Mutation screening in an independent cohort of 144 T-ALLs identified additional mutations, resulting in total mutation frequency of 3.8% (8/211). Of interest, we found that *CNOT3* mutations were significantly more frequent in adult T-ALL cases (7.9%; 7/89; $p<0.01$).
To investigate the effect of loss of *CNOT3* in tumour formation, we used an established *Drosophila melanogaster* eye cancer model. We used the 'sensitised' model in which the *Notch* ligand *Delta* is overexpressed in the developing eyes. These flies have larger eyes, but by themselves do not develop tumours. Given the central role of NOTCH1 signaling in T-ALL (activation of NOTCH1 receptor is estimated to participate in more than 50% of T-ALLs) this model is relevant for our study. Besides, loss of *Not3* expression in the sensitised eye discs results in the disruption of the regular pattern of the retinal epithelium. These data support that a reduction of *Not3* expression is sufficient to transform sensitised cells.
In conclusion, we found that *CNOT3* was mutated in adult T-ALL. Our experiments in a *Drosophila* cancer model suggest that the *CNOT3* gene could act as a tumour suppressor in T-ALL.

P.10 Role of HH pathway in T cell acute lymphoblastic leukaemia (T-ALL)

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T cell acute lymphoblastic leukaemia (T-ALL) is a neoplastic disorder that occurs in 15% of pediatric and 25% of adult acute lymphoblastic leukaemias. T-ALL is a genetically complex disease in which various genetic changes have been identified, including chromosomal translocations activating TLX1 or TLX3, CDKN2A/ARF deletions, and activating mutations in NOTCH1, PHF6, IL7R or JAK3. Nevertheless, despite the accumulative knowledge obtained over the last years additional genetic alterations remain to be discovered. The identification of factors that contribute to the malignant behavior of these leukemic cells could subsequently lead to the development of optimal biomarkers to predict prognosis in T-ALL patients.
The Hedgehog (Hh) family of secreted proteins governs a wide variety of processes during embryonic development and adult tissue homeostasis. It has been shown, that hyperactive Hh signaling plays an important role in solid tumours like breast cancer, medulloblastoma and basal cell carcinoma. More recently, it has been reported that aberrant Hh signaling is important for stem cell maintenance and contribute to resistance to apoptosis of leukemic cells. However, mutation or activation of the Hh pathway in T-ALL has not been described.
By performing sequencing in fifty T-ALL patients we identified four mutations in Hh pathway components SMO, GLI1 or GLI3 (8% frequency). Furthermore, pharmacological inhibition of Hh pathway led to reduction of proliferation of T-ALL cell lines. The requirement of the Hh pathway for T-ALL cell proliferation was confirmed with siRNA knock-down studies in cell lines. In agreement with this, knock-down of SUFU, a negative regulator of the Hh pathway, caused resistance to Hh pathway inhibitors.
These data indicate an important role for Hh pathway in T-ALL development and suggest that Hh inhibitors should be explored for T-ALL treatment.

P.11 Expression of ZAP-70 in CLL activates NF-?B signaling

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Chronic lymphocytic leukaemia (CLL) is a disease with a highly variable prognosis. The clinical course can however be predicted thanks to prognostic markers. Poor prognosis is associated with expression of a B cell receptor (BCR) from unmutated immunoglobulin variable heavy chain genes (IgVH) and expression of zeta-associated protein of 70 kDa (ZAP-70). The reason why ZAP-70 expression is associated with poor prognosis and whether the protein has a direct pathognomonic function is at present unclear. We wished to further explore this mechanism.
By mRNA elektroporation we transferred ZAP-70 into CLL cells and compared ZAP-70 negative cells versus ZAP-70 positive ones. We also stimulated the cells with IgM and measured Ca flux, cytokine production. We used cells collected in eight patients being negative for ZAP-70.
Expression of ZAP-70 in CLL cells leads to increased expression of the NF-?B target genes interleukin-1 (IL-1), IL-6 and IL-8 upon BCR triggering. This could be blocked by inhibition of NF-?B signaling through inhibition of I?B kinases (IKK). Transcriptome analysis identified a NF-?B RelA signature imposed by ZAP-70 expression in BCR stimulated CLL cells.
We conclude that ZAP-70 acts directly as an amplifier of NF-?B signaling in CLL cells which could be an underlying mechanism for its association with poor prognosis.

P.12 Evaluation of CD200 expression in differential diagnosis of CD5+ B cell NHLs

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Introduction
In CD5+B-cell neoplasms differential diagnosis between CLL/SLL, PLL and MCL is based on immunophenotyping (IF), morphology and cytogenetics (FISH/karyotyping). A Catovsky score >3/5 is regarded as CLL whereas <1.5/5 is suggestive for MCL, PLL or CLL with atypical presentation. Exclusion of atypical CLL as a morphological entity (WHO2008) has increased the risk for false MCL suggestions based on IF alone. Recently, CD200 emerged as a useful differentiating marker with strong expression in CLL but absence in MCL. Since t(11;14)(q13;q32) with cyclinD1 (CCND1) over expression is harbored by virtually all MCL, we evaluated CD200 expression in CCND1 rearranged and non-rearranged CD5+B cell NHLs.

Material and methods
Thirteen cases suggestive for MCL (CD5+ and Catovsky-score <1.5/5) were investigated for CD200 expression (FACSCantoII,BD), with FISH detection of CCND1 rearrangement (dual color break apart probe,Vysis,Abbott) serving as golden standard. Non-rearranged cases were subsequently analyzed for trisomy12, del(13)(q14), del(17)(p53) and del(11)(q23), recurrently involved in CLL. We compared the median %CD200 positivity of B-lymphocytes and fluorescence intensity (MFI) among all thirteen cases and compared these results with five typical CLLs (Catovsky-score = 4/5).

Results
CCND1 rearrangement was detected in 5/13 cases, all sharing negative CD200 expression. The remaining 8 CCND1 non-rearranged cases showed two subgroups, either with (n=4) or without (n=4) CD200 expression. FISH and karyotyping results in the CD200 positive subgroup were compatible with CLL diagnosis. Similarly, FISH on CD200 negative subgroup together with cytologic features, lead to final diagnosis of PLL(n=1) and CLL with atypical presentation (n=3). The median %CD200 positivity and MFI for CCND1 rearranged *vs* non-rearranged cases (4.3% *vs* 16.7%,243 MFI *vs* 283 MFI) seems to overlap, but both subgroups strongly differ from the CD200 positive CCND1 non-rearranged cases (98.2%,MFI 3726) and typical CLLs (99.7%,MFI 5962).

Conclusion
Absence of CD200 cannot be used as a surrogate marker for t(11;14)(q13;q32) given the lack of CD200 expression in 4/8 CCND1 non-rearranged case. On the other hand, CD200+ expression in our cases definitively excludes MCL diagnosis.

P.13 G8 as a screening tool for older patients with malignant haemopathies: A surrogate for CGA?

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Background
Growing evidence suggests that a Comprehensive Geriatric Assessment (CGA) could help haematologists to detect unsuspected health problems, to estimate survival and to predict tolerance in order to optimise treatment. Nevertheless, it is time consuming and expensive. Short-screening tool (G8) could allow screening for fragile patients but requires validation in malignant haemopathies.

Aims
To evaluate the respective usefulness of G8 and CGA to predict survival in a selected population of fit older patients referred for the treatment of malignant haemopathies. To assess the usefulness of G8 to predict the initial treatment choice and the tolerance to chemotherapy.

Methods
Between October 2009 and September 2012, a G8 and a full CGA assessment were proposed to 77 older patients (=65 years) considered fit enough by referral physicians to receive chemotherapy. The normal cut-off for G8 assessment has been defined =15/17. Patients' initial treatment choice, tolerance to chemotherapy, death and causes of death were extracted from medical files.

Results
We assessed 68 patients. G8 screening score was abnormal in 80% of the patients. Difference between normal and abnormal G8 scores did not influence initial treatment choice (p=0.495), tolerance to chemotherapy (p=0.300) and is not associated with one year survival (p=0.635). CGA showed that a probable mild cognitive disorder (MMSE) (p=0.031) is associated with a higher risk of death in the first year of treatment.

Conclusions
Among older patients with haematological malignancies, the G8 screening identifies very fit patients but is not specific enough to select older patients susceptible to benefit from full dose chemotherapy. In our small series of fit older patients, a poor G8 score does not allow to predict tolerance to chemotherapy and does not translate into a worse survival. Prospective trials are needed to determine whether G8 score with specific cognitive items could be adapted to malignant haemopathies.

P.14 Bendamustin shows distinct anti-tumoural responses in multiple myeloma patients that relapsed after prior bortezomib and lenalidomide treatment. A study on behalf of the MM BHS subcommittee

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Bendamustin - an alkylating agent with purine-analogue like activities - exerts high efficacy in B-cell malignancies. It is active against multiple myeloma (MM) cell lines, as well as in both de novo and relapsed/refractory MM patients (patients) A Belgian Medical Need Program (MNP) was initiated from February till October 2012 and included twenty patients. Inclusion criteria consisted of: 1. relapse (refractory ?) after both Bortezomib and Lenalidomide based therapy; 2. a good performance score (ECOG 0-1); 3. absence of end-stage renal disease, 4. correct residual marrow function and absence of plasma cell leukaemia.

We here report the results of Bendamustin therapy in 16 patients enrolled in this MNP. Median age was 69 years (57-79) with a median number of 4 (3-8) lines of prior therapy. The median number of Bendamustin cycles infused was 4 (1-5). The initial dosage varied from 70 to100 mg/m2, d1 and d2 every 4w, according to physician's choice. A total of 58 cycles of Bendamustine were delivered. Adverse side effects were mainly hematological toxicity. The overall response rate (ORR) (evaluated according to EBMT criteria) was 50% (eight patients, one very good partial response, six partial responses and one minor response) and 31% of patients experienced stable disease.

In this heavily pretreated population, response rates seem encouraging and are concordant with responses reported in the literature. We believe that well-selected double refractory patients (as proposed by our inclusion criteria) might benefit from bendamustin as salvage treatment.

P.15 Genomic profiling of myeloma: A comparison of cytogenetics, FISH and array- CGH analysis of 95 cases at diagnosis or follow up

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Cytogenetic and FISH assays play pivotal roles in the risk stratification of newly diagnosed myeloma. Major prognostic indicators include presence of a karyotypically abnormal clone, presence of chromosome 13 abnormality by metaphase analysis and FISH detection of a t(4;14), t(14;16) or deletion P53. According to the International myeloma working group FISH analysis for deletion 17p, t(4;14) and t(14;16) is the priority test for this neoplasm, with further optional analyses for hyperdiploidy,13q-, loss1p/gain1q, t(11;14). More recently, loss of 12p has been added and it is expected that this list will increase as new prognostic markers are identified by array analysis. It is recommended where possible to complement FISH analysis with cytogenetics and CGH analysis. Array CGH is a relatively new technique which provides a genomic profile of gains and losses and is particularly useful for non proliferative or poorly proliferative disorders such as myeloma. Using this approach genomic imbalances are detected in virtually all cases and to a large extend this approach can replace traditional cytogenetic and FISH tests.

We carried out a comparative study of 95 myeloma cases analysed by cytogenetics, FISH and microarray at diagnosis, or at follow up, with view to rationalising genetic testing of this pathology. Genomic abnormalities were detected in 25% by cytogenetics, and in 93% by FISH and microarray. Overall, there was a good concordance between the different techniques. Array CGH provided a more comprehensive overview of genomic aberrations than either cytogenetics or FISH. FISH and microarray did not always correctly identify ploidy compared to cytogenetics however, when compared to FISH, array CGH analysis did correctly identify the ploidy group even if it could not make the distinction between 1n, 2n and 4n clones. Microarray analysis did not always detect low level aberrations identified by FISH.

Whilst array CGH may in the future replace conventional cytogenetics and certain FISH tests complementary FISH analysis is required for the identification of the recurrent translocations associated with this disease.

P.16 Expression of TMEM45A, a candidate gene of chemoresistance, in a panel of neoplastic tissues

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Background

The development of cancer cell resistance against chemotherapy limits the efficacy of most current anticancer treatments. Although mechanisms of resistance are partially understood, the most important mechanisms remain unknown. Hypoxia, a common feature of the tumour environment, has been shown to play a role. Recently, Flamant *et al* (BMC Cancer, 2012) showed that TMEM45A, a transmembrane protein, was essential for hypoxia-induced chemoresistance in breast and liver cancer cells and that its expression level could be used as a molecular prognostic marker to identify a potential resistance to chemotherapy in breast cancer.

Aim

In order to validate TMEM45A as a marker of chemoresistance, we first looked at its expression level in a panel of haematologic and non-haematologic neoplastic tissues.

Methods

The RNA expression level of TMEM45A was quantified by real time PCR using the threshold cycle method. The results were compared to its expression in corresponding healthy tissues.

Results

The expression of TMEM45A was enhanced in most of tumour tissues compared to healthy tissue especially in epidermod tumours. A similar differential expression was found in haematologic samples (leukaemias, myelomas and lymphomas) but in this case the expression of TMEM45A remained particularly low except for myeloma samples.

Conclusion

Our results suggest that TMEM45A might participate to chemoresitance mechanisms in a large panel of tumours. In the hematological context, TMEM45A expression was globally low except for myeloma, a tumour where the hypoxic niche of the bone marrow is particularly important for the survival of the cells. However, its role in chemoresistance remains to be determined. This work also enhances the importance of biobanking.

Perspectives

The results need to be confirmed on a larger number of samples. We are currently quantifying TMEM45A expression in samples collected at different time-points of the treatment (diagnostic, resistance, relapse).

P.17 Complete responses of the TEMPI syndrome to bortezomib

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We recently described the TEMPI syndrome (1), which is characterised by the pentad of telangiectasias, elevated erythropoietin level and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting. One of the patients had a dramatic response to treatment with the proteasome inhibitor bortezomib, and we hypothesized that the paraprotein may play a role in the pathophysiology of the TEMPI syndrome.

A 48-year old woman, received a total of eight cycles of intravenous bortezomib (four doses of 1.3 mg per square meter of bodysurface area per cycly). Her telangiectasias disappeared (Fig. 1, Panels A through D), her perinephric fluid collections disappeared (Fig. 1E and 1F), and her serum levels of erythropoietin decreased from 6400 mIU per millimeter to 19 mIU per millimeter. Levels of IgG kappa paraprotein became undetectable. Before treatment, she required a wheelchair and continuous supplemental oxygen; since the completion of treatment, her intrapulmonary shunting has resolved and she has resumed jogging. She remains in complete remission eighteen months after receiving het last dose of bortezomib.

The efficacy of bortezomib treatment, as well as the completely reversible nature of the symptoms, suggests that the abnormal plasma-cell clone and monoclonal gammopathy are the likely cause of the TEMPI syndrome. Efforts to identify the antigenic target of

the paraoprotein are under way. We suspect that there exist other patients with the TEMPI syndrome - as well as patients with other disorders - whose symptoms might be explained by a plasma-cell dyscrasia or underlying monoclonal gammopathy.

P.18 Concurrent B-cell chronic lymphocytic leukaemia and multiple myeloma

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The concurrence of two distinct B-cell malignancies in one patient is uncommon and the origin of the malignant clone(s) remains controversial. The therapeutic challenge in these patients is to find an effective treatment for both diseases. We present two patients with concurrent CLL and MM, treated with lenalidomide.
The first patient (male, 61y) had concurrent B-CLL Binet B and IgA-k MM ISS I. PET revealed multiple adenopathies, without osteolytic lesions. A biclonal peak IgA (20,4 g/L) and IgG (5 g/L) was found. Marrow aspiration showed a central lymphocytosis (40%)(CD5+, CD20), and 28% k-monotypic plasma cells (CD19-,CD56+) with cytoplasmic IgA. Lymph node biopsy revealed complete infiltration by a small lymphocytic lymphoma. Lenalidomide in medical need was started. After 4 cycles, bortezomib was associated because of suboptimal response. Re-evaluation showed a near CR of both MM and CLL with 1,2% central plasma cells and 0,03% lymphocytes of the original phenotype and marked reduction of cervical and absence of other adenopathies. Consolidation with autologous stem cell transplantation is planned.
The second patient (female, 79) has a history of breast carcinoma, treated with surgery and RT. B-CLL was diagnosed seven years ago and never required treatment. Recently, she presented with sternal pain due to a plasmocytoma. A monoclonal peak IgA-? of 7.19 g/L was seen, with suppression of the uninvolved immunoglobulins. There was an abnormal sFLC ratio of 0.21 (0.26-1.65). Immunophenotyping of the marrow aspirate showed only 2 B-CLL populations. Both had a typical CLL signature, but one population had a higher expression of CD5. On bone marrow biopsy, besides numerous CD5+/CD23 lymphocyte aggregations, large focal infiltrates of -monotypic plasma cells, compatible with MM were found. The sternal plasmocytoma was treated with local RT and lenalidomide in compassionate use was started with promising result.
In conclusion, we report two patients with concurrent B-CLL and MM, in whom we started treatment with lenalidomide, with promising results on both malignant clones.

P.19 Clonaly related monoclonal B-cell lymphocytosis and diffuse large B-cell lymphoma in a patient treated for EBV-positive Hodgkin lymphoma

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Case report
A previously healthy HIV-negative 32-year-old male was diagnosed with an Epstein-Barr Virus (EBV)-positive classical (mixed cellularity) Hodgkin lymphoma (HL), stage IIIB. Simultaneously, a small monoclonal cell population with a mature B-phenotype, was evidenced in blood and bone marrow. However, immunoglobulin gene rearrangements were different from those observed in the HL-positive lymph node. Treatment consisted of six courses of ABVD, resulting in a complete remission of the HL. In addition, the monoclonal B-lymphocytes were no longer detectable.
After four years of continued remission, the patient presented with an aggressive EBV-positive diffuse large B-cell lymphoma (DLBCL), stage IVBE (pericardial involvement). Immunoglobulin gene rearrangements performed on several tumour samples were identical to those established in the circulating monoclonal cells at primary diagnosis of HL. He was treated with intensive chemotherapy followed by BEAM and autologous stem cell rescue, yielding a complete remission. One year later, an EBV-positive DLBCL relapse was documented. Treatment consisted of chemotherapy followed by a reduced intensity conditioning MUD allogeneic stem cell transplantation. However, the patient was rapidly progressive and died of CNS involvement.

Discussion
This case clearly illustrates that an apparently indolent monoclonal B cell population can evolve directly into an aggressive DLBCL, several years later. Among possible contributing factors in our patient are: ABVD chemotherapy, secondary immunodeficiency and EBV infection. In addition, the presence of EBV, demonstrated in both HL and DLBCL samples, is likely to reflect the contribution of EBV infection to the lymphomagenesis in this patient. Finally, this case supports to specifically target co-existent B-cell lymphoproliferations in patients with HL, e.g. by the use of monoclonal antibodies such as rituximab.

P.20 Primary Intraocular Lymphoma: a Case Report

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Introduction
Primary Intraocular Lymphoma is considered a subset of Primary Central Nervous System Lymphoma (PCNSL), usually of the non-Hodgkin Diffuse Large B-cell (DLBCL) type and is associated with a poor prognosis.

Case Report
A 66-year old woman was seen, complaining of progressively diminishing vision. Fundoscopy demonstrated bilateral signs of vitritis, variable white dots and retinal pigment epithelial. A temporal artery biopsy was negative and brain MRI showed no orbital or intracranial lesions. With a tentative diagnosis of white dot syndrome, the patient was started on an immunosuppressive dose of corticosteroids which led to a subjective improvement. On cessation however, her visual symptoms relapsed and a diagnostic vitrectomy was eventually done with a five-month delay.
Cytopathology revealed DLBCL. Further staging with brain MRI and full body CT/PET-scan showed an additional lesion in the cerebral occipital region.
Two cycles of chemotherapy were conducted in accordance with the EORTC-protocol for the treatment of PCNSL, simultaneously given with intraocular (i.o.) MTX, methylprednisolone and Rituximab. An evaluation with PET/CT and MRI showed no signs of tracer activity and involution of the lesion, respectively. A consecutive second identical cycle was administered but no additional whole brain radiotherapy (WBRT) was given.
During treatment only limited side-effects were noted and a spectacular improvement of vision was obtained within a matter of weeks. Our patient is currently still under ambulant observation, with a clinically disease free period of almost two years.

Discussion
The diagnosis of PIOL needs to be considered in any patient with signs of posterior uveitis and CNS symptoms. It is a diagnosis difficult to make and delays up to 21 months have been reported.
Treatment consists of i.v. chemotherapy with HD-MTX being a cornerstone in the therapeutic management of PIOL. Administration of intraocular MTX has also become a standard in the management of retinal lymphoma, whereas the additional role of intraocular rituximab is still under investigation.

P.21 Coincidence of renal cell carcinoma and post-transplant lymphoproliferative disorder following kidney transplantation

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Posttransplant malignancy (PTM), caused by iatrogenic immune suppression, is a serious complication of solid organ transplantation. One of the most common cancers after kidney transplantation is posttransplant lymphoproliferative disorder (PTLD).
We report a case of a 38-year-old patient who presented with a papillary renal cell carcinoma of a native kidney twelve years following second kidney transplantation. After bilateral nephrectomy, a CT and MRI abdomen showed several liver lesions. Radiological findings were suggestive for metastases for which systemic therapy was planned. However, pathological examination of a liver biopsy surprisingly showed the presence of EBV-negative PTLD, subtype diffuse large B-cell lymphoma. Further staging with PET-CT scan revealed diffuse liver- and bone involvement. Bone marrow examination was unremarkable. Treatment with reduction of immunosuppression (RIS) and four courses of Rituximab (375mg/m weekly) was initiated. Re-evaluation with PET-CT scan showed only one persisting FDG-avide lymph node abdominal. A new biopsy confirmed PTLD. As the patient was considered to be in complete remission following RIS, Rituximab and surgery, maintenance therapy with four additional courses of rituximab was given. More than two years following this therapy, the patient is in persistent complete remission. Unfortunately, his renal function has evolved to preterminal kidney failure due to chronic allograft nephropathy for which he is on the waiting list for a third kidney transplantation. This case underscores the increased susceptibility to PTM in transplant patients. Diagnosis with excision biopsy is of paramount importance, both in the initial diagnostic work-up as in case of doubt in re-evaluating the patient. Furthermore, this case illustrates the potential role of PET-CT scan in the diagnosis and staging of PTLD and the possibility of re-transplantation in patients with prior diagnosis of posttransplant malignancies.

P.22 About two cases of Merkel cell carcinoma associated with chronic lymphocytic leukaemia

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Merkel cell carcinoma (MCC) is an aggressive primary skin tumour mainly occuring in the elderly. This kind of tumour is frequently associated with another neoplastic disease. The second neoplasm can be either a solid tumour, usually of skin origin, or an haematological malignancy, usually a B-cell chronic lymphocytic leukaemia (CLL). We report two consecutive cases diagnosed with both neoplasms.
A 63-year-old male presented with a red cutaneous lesion of the right buttock area during the second course of FCR immune-chemotherapy for a Binet stage B CLL. Biopsy confirmed the diagnosis of MCC, further staged as TNM stage 3B. Further treatment included surgical resection of the skin lesion and invaded inguinal lymph nodes. Adjuvant radiotherapy is still ongoing.

A 73-year-old female with a Binet stage C CLL developed a red cutaneous lesion of the right tight, proven to be a MCC, TNM stage 3A, 3 months after the initiation of a chemotherapy with chlorambucil. Surgical resection was followed by local radiation therapy. However, the patient developed a Richer's syndrom and achieved a complete remission after eight courses of R-CHOP. Unfortunately, she presented a first relapse of MCC with three lesions of the left leg which were totally resected, and a second relapse on the right paravertebral area. Despite chemotherapy as well as radiation therapy on the paravertebral metastasis, she died from disease progression.

The association of MCC with CLL is now well recognized. Patients diagnosed with CLL have a 15.7 fold increased risk to develop MCC. Immunosuppression induced by the haematological disorder certainly plays a role in the pathogenesis of MCC, as well as a possible Merkel-cell polyomavirus infection.

P.23 Heavy/light chain (HLC) and free light chain (FLC) analysis allow sensitive monitoring of multiple myeloma patients and aid detection of clonal changes

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Background
Traditional tests to monitor monoclonal intact immunoglobulin (M-Ig) components in multiple myeloma (MM) include SPE, IFE and free light chains (FLC). Magrangeas *et al*. have reported that at relapse in 66% of patients there is a change in predominant plasma cell clone. Nephelometric assays have been developed that measure Ig'?/Ig'? (heavy/light chain; HLC; Hevylite™) and FLC (Freelite). Here, we present two case studies demonstrating HLC/FLC analysis aid detection of clonal changes at relapse.

Methods
IgAk,IgA?,FLCk,FLCl were measured nephelometrically using HLC and FLC, respectively. Results were compared with retrospective SPE, IFE and total IgA (TIgA).

Case 1
An IgA? MM patient presented with 31g/L monoclonal IgA? (measured by TIgA as a surrogate marker), abnormal HLC IgAk/? ratio (0.33) and FLCk/? ratio (0.14). The patient underwent MPV therapy from day 29-159. TIgA, HLC ratio and HLC IgA? demonstrated therapy successfully reduced the monoclonal IgA? expressing clone, with the patient achieving a very good partial response (VGPR) at day 137, which was maintained until day 202. In contrast, between days 29-202 there was a progressive increase in FLCk/? ratio. Between days 202-277, MPV maintenance therapy successfully reduced FLC? expression. However, at this time TIgA and HLC IgA? levels indicated re-emergence of an IgA? expressing clone.

Case 2
An IgAk MM patient expressed monoclonal IgAk and FLCk. At day

0 the patient presented an abnormal HLC (75) and FLC ratio (203). After 331 days, following 4 cycles of VAD induction therapy and ASCT, the patient achieved a stringent complete response; HLC ratio normalised at this point. From day 331-549 HLC ratio, HLCk levels and SPE indicated the therapy had reduced the primary IgAk expressing-clone. However, from day 338 there was a significant increase in FLCk and FLC ratio, indicating emergence of a secondary clone which predominantly expresses FLC.

Conclusions
HLC ratios offer a sensitive alternative to traditional measurements for the monitoring of MM patients, and in conjunction with FLC ratios offer a serum based measure of clonal changes.

P.24 Neurolymphomatosis: 2 cases diagnosed with FDG PET.

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Neurolymphomatosis (NL) is lymphomatous infiltration of single or multiple cranial and peripheral nerves and nerve roots.
A 39-year old female with diffuse large B-cell lymphoma of the bone achieved a complete remission after 6 cycles of R-CHOP14. The treatment was consolidated with BEAM and autologous stem cell transplantation. One month later she presented with acute onset of L5 radiculopathy. Brain MRI and cerebrospinal fluid tested negative. However FDG PET scan showed unilateral involvement of the radices L5 and S1. MRI of the lumbar spine demonstrated an intense contrast uptake of the same nerve roots highly suspicious for lymphomatous radiculomeningitis. Although radiation therapy gave immediate relief, NL progressed rapidely to the cranial nerves with unilateral abduscens pareses. Cerebral fluid revealed a monoclonal B-cell population and she was treated with high dose Methotrexate. Nevertheless condition deteriorated and patient opted for palliative care.
A 48-year old male was diagnosed with a mixed small and large cell lymphoma with prominent extranodal disease in the bone, pericardium, pleura and cerebral fluid. After 3 cycles of R-CHOP and intrathecal administration of Depocyte he achieved a complete metabolic remission on FDG PET scan and the CNS fluid tested negative. After the 5th cycle however he presented with progressive loss of vision. Restaging confirmed complete remission and he was treated for Depocyte-induced chemical arachnoiditis with high dose corticosteroids. After initial improvement he became completely blind and condition deteriorated with progressive paresis and paralysis of the facial nerves, upper and lower limbs. FDG PET showed diffuse tracer uptake of brachial and lumbosacral nerves and plexuses. Guillain barr variant was suggested and high dose of immunoglobulins was intitiated without success. CNS fluid retested positive for monoclonal B-cells and high dose of Methotrexate was administered. However condition deteriorated and the patient died. NL remains difficult to diagnose since it may mimic many conditions. These cases illustrate the utility of FDG PET-CT in establishing the diagnosis of NL.

P.25 Unusual isolated intracranial relapse of multiple myeloma

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Intracranial involvement is a rare extramedullary complication in multiple myeloma (MM), resulting from osteodural plasmocytoma or leptomeningeal involvement. Primary parenchymal brain lesions have been described sporadically. They are usually part of a systemic disease although solitary tumours with no sign of MM have been reported.
A 43-year-old man presented with dyspnea and diffuse edema in April 2011. Peripheral blood showed 23% of plasma cells (PC). Chest X-ray displayed bilateral pleural effusion, and the fluid contained PC. Fluorescent *in situ* hybridization performed on PC showed a non hyperdiploid status without 14q32 nor 17p aberrations, as well as trisomy 1q21. Serum protein electrophoresis and immunofixation revealed a monoclonal IgD-lambda component. Bence-Jones proteinuria and renal insufficiency (creatinine 5.05 mg/dL) were observed. Spinal MRI showed diffuse bone marrow infiltration. The patient was diagnosed with plasma cell leukaemia (PCL)
Initial treatment consisted of 5 cycles of bortezomib-dexamethasone and cyclophosphamide. A complete response was achieved with normal serum and urine immunofixation and recovery of the renal function. Further therapy included high dose melphalan (200 mg/m) and autologous stem cell transplantation in August 2011. Restaging of the PCL at day 100 post-transplant showed absent disease. He received three consolidation courses of bortezomib and dexamethasone.
In November 2012, he presented with dizziness, hemiparesis and visual impairment. Brain MRI showed multiple intraparenchymal enhancing lesions with edema. Because of intra-cranial hypertension, lumbar puncture was not carried out. Bone marrow biopsy showed no PC infiltration. Skeletal lesions were not identified. Neurosurgical stereotactic biopsy confirmed the diagnosis of plasmocytoma. Immunohistochemical stains showed ? chain restriction.
Infiltration of cerebral parenchyma by MM, without contiguous bone lesions, following ASCT is very uncommon. The origin could be haematogenous, explaining why PCL is considered a risk factor. The prognosis is poor, with a median survival in months. No therapeutic guidelines have been established. The activity of new agents (i.e. lenalidomide) and their ability to cross blood-brain barrier are largely unknown. These agents may also predispose patients to extramedullary plasmocytomas.

Abstracts posters myeloid malignancies (P.26-P.46)

P.26 Meningioma 1 (MN1) expression: Refined Risk Stratification in acute myeloid leukaemia with normal cytogenetics

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Several novel prognostic factors have improve prognostic stratification of patients with cytogenetically normal acute myeloid leukaemia (CN-AML). The aim of this study was to determine the prognostic importance of the meningioma 1 (MN1) gene expression levels in CN-AML. One Hundred patients with AML were diagnosed, MN1 expression were analyzed using quantitative real time (QRT) PCR. High expression was detected in 48 (48%) patients (range: 2.35-31.99, mean: 13.9 8.49) in comparison to 52 (52%) patients with low expression(range: 0.02-2.3, mean: 0.68 0.77). The course of the disease in patients with high MN1 expression was unfavorable. Patients with high MN1 expression was associated with significant low complete remission (CR) rate (62.5% *vs.* 88.4%, high *vs.* low MN1, P =0.001) and high mortality rate (75% vs 46.1, *P*=0.03).AML patients with high MN1 expression tended to be refractory (37.5% *vs*

19.2, *P*=0.00) and relapse risk (54.1% *vs* 23%, *P*=0.02). Multivariable analysis confirmed high MN1 expression as an independent risk factor for disease free survival (DFS) and overall survival (OS). In Conclusion, MN1 over expression independently predicts bad clinical outcome in CN-AML patients. MN1 overexpression is associated with poor induction response, shorter relapse-free survival, and shorter overall survival. This leads to improve risk stratification of this heterogeneous group of patients with AML.

P.27 Invariant Natural Killer T cells in the 5T33 multiple myeloma model

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Invariant Natural Killer T cells (iNKTs) are T-lymphocytes co-expressing T and NK receptors. It has been described that iNKTs have an anti-tumour activity through the activation of their unique TCR a-chain by a-Galactosylceramide (a-GalCer) in the context of CD1d molecules. Therefore, stimulating iNKTs in the 5T33 multiple myeloma model can be a useful preclinical tool.

We followed the frequency of iNKTs during the development of the disease in both 5T33MM mice and MM patients and found that their numbers declined dramatically at the end stage of the disease (from 7% to 2.6% in mice liver cells and from 737 to 76 iNKT/ml in blood of relapsed patients). We analysed the activity of these iNKTs through IFN? secretion by co-culturing liver iNKTs with a-GalCer loaded DCs. We found that IFN? dropped from 2.3 ng/ml to un-detectable levels at end stage of the disease which is the result of the decline in iNKT number and not through internalization of their Va14 receptors as determined by RT-PCR.

We investigated the response of iNKTs to a-GalCer *in vivo* and found that the IFN? response in non-terminal diseased mice was equal to that measured in naive mice, confirming the possibility of inducing Th1 responses with a-GalCer. We furthermore found that a-GalCer treatment significantly increased the survival of 5T33MM mice from 22 to 29 days.

To examine whether a-GalCer activation of iNKTs has an effect on tumour angiogenesis, 5T33MM mice were treated with a-GalCer at 4 day intervals. A significant decrease of the microvessel density (MVD) was observed in the treated group (23.7%) compared to untreated group (33.3%), almost back to naive levels (19%). This was independent of the reduction in tumour load. Rat aortic ring assays were performed to confirm that IFN? mediates the inhibition of angiogenesis.

These data demonstrate for the first time the possibility of using a preclinical MM model to study the effects of a-GalCer on iNKTs and shows promising results of treating early-stage MM patients.

P.28 The IGF-1 receptor inhibitor picropodophyllin (PPP) potentiates the anti-myeloma effects of the BH3 mimetic ABT-737

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The role of insulin-like growth factor 1 (IGF-1) in the pathophysiology of multiple myeloma (MM) has been well established thereby forming an attractive target. Although we previously demonstrated that the IGF-1R inhibitor picropodophyllin (PPP) has potent anti-MM effects, treated MM bearing mice eventually relapsed. To overcome this relapse, combining PPP with other cytotoxic drugs is an attractive approach. The BH3 mimetic ABT-737 shifts the survival/apoptosis balance toward apoptosis by binding to the pro-survival proteins Bcl-xL and Bcl-2, but not to Mcl-1. In MM, elevated expression of Mcl-1 has extensively been shown to contribute to drug resistance. Consequently, ABT-737 has potent anti-MM activity but only on a subset of human cell lines. Interestingly, a protective effect of IL-6 and bone marrow stromal cells on the ABT-737-induced apoptosis was reported. This suggests that it would be beneficial to combine ABT-737 with PPP. Here, we investigated the combination of ABT-737 and PPP in human MM cell lines and in the murine 5T33MM model. Both agents alone were found to decrease viability and induce apoptosis, dose and time dependently. Moreover, combination of both agents synergistically decreased cell viability and induced apoptosis compared to single agent treatment. Preliminary results also confirm this synergistic anti-MM activity in primary human MM samples. Mechanistically, western blot analyses revealed that combination treatment results in enhanced cleavage of caspase 3, 9 and PARP, increased expression of the pro-apoptotic protein Noxa and reduced expression of Mcl-1 and Bcl-2. Moreover, while the CD138+ 5T33MM subpopulation was more sensitive to PPP and the CD138- cells more sensitive to ABT-737, both subpopulations were targeted equally when treated with the combination. Lastly, the 5T33MM model was used to evaluate the *in vivo* anti-MM activity of PPP and/or ABT-737 in prophylactic setting. Combinatory treatment significantly decreased BM tumour burden and prolonged overall survival of the combination treated mice (*p*=0.001). In conclusion, the combination of PPP and ABT-737 has synergistic anti-MM activity and may thus be a promising treatment option for MM.

P.29 The multifaceted GATA2 gene in inherited myeloid malignancies: about a Belgian family

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Rare familial cases of myelodysplastic syndrome (MDS) / acute myeloid leukaemia (AML) are reported in which five disease genes have been identified to date : *RUNX1, CEBPA, TERC, TERT* and, more recently, *GATA2*. The phenotypic expression of *GATA2* mutations (autosomal dominant transmission) may include dendritic cell, monocyte, B- and NK-cell deficiency and / or congenital lymphoedema (Emberger syndrome) with no obvious genotype / phenotype correlation, suggesting that these clinical presentations

are a continuum of the same disease.
We report a new heterozygous nonsense *GATA2* R330X mutation identified in four men within a Belgian family. Two brothers exhibited extensive warts and mild neutropenia preceding AML or MDS at 17 or 19 yr-old respectively. Both myeloid malignancies were associated with an acquired der(1;7)(q10;q10) leading to a monosomy 7q and a trisomy 1q. The older brother died from *Aspergillus* infection during the induction therapy. The second brother remains in remission two years after hematopoietic stem cell transplantation (HSCT) with an unrelated donor. The third brother (20yr-old) only exhibited mild neutropenia, thrombopenia and B-cell deficiencies and the father only B-cell deficiencies. The paternal uncle, although not tested, is suspected of having a *GATA2* mutation (extensive warts associated with mild neutropenia and monocytopenia). He suddenly died at the age of 36 due to an aortic dissection.
The analysis of the French national registry of chronic neutropenia allowed the identification of six additional pedigrees with six different and not previously reported *GATA2* mutations. The frequent evolution to MDS and AML of these patients reveals the importance of screening *GATA2* in chronic neutropenia associated with monocytopenia and / or mild B- or NK-cell deficiency due to their frequent hematopoietic transformation, their variable clinical expression at onset and the need of aggressive strategy therapy (HSCT) in patients with poor clinical outcome.

P.30 Acute myeloid leukaemia infiltrating-T lymphocyte characterization

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We have assessed quantitatively and qualitatively the purified T cell population from AML patients, using flow cytometry and Affymetrix microarray studies, at diagnosis and in complete remission, in bone marrow and peripheral blood samples, in an effort to better correlate the role of the absolute number and percentage of the various T-cell subpopulations to the outcome of the disease in terms of relapse-free survival and overall survival, in otherwise undistinguishable leukaemia as far as known prognostic factors are concerned. Unsupervised analyses revealed important significant differences between leukemic patients and healthy individuals in the gene expression profile of their T-lymphocytes. To better understand the dissimilarities between the different samples and validate microarrays data, we have also performed an analysis of the cytokine mRNAs produced by infiltrating T-cells, using quantitative RT-PCR arrays (Human Cytokines, Chemokines and Receptors StellARray qPCR array, T Regulatory Phenotyping StellARray qPCR Array) from LonzaTM. T-cell polarization bias in AML patients *vs* healthy individuals consist in the fact that type 1 T-cell response associated molecules are downregulated, type-2 T-cell response associated molecules are upregulated, regulatory T-cell associated molecules are upregulated, innate immunity is inhibited, immuno-suppressive molecules are expressed, T-cell activation, inflammation and immune cell recruitment genes are expressed. Another objective was to determine whether inter-individual differences in the immune microenvironment had an impact on the outcome of the disease, in a given patient subgroup, having an identical prognosis when using the classical approaches currently in use. We could observe patient subgroups with distinct immune signature. Most of AML studies were focused on the leukemic blast biology, but circulating immune cells in patients seem to reflect an important message. Further study of leukaemia microenvironment is needed to understand its role in this pathology and maybe reveal new possible therapeutic approaches or more patient specific therapy. In its current status, this study suggests the immediate possibility of initiating clinical studies aiming for example at inhibiting Tregs after chemotherapy for patients not assigned to allogeneic HSCT.

P.31 Study of the miRNome of childhood myelodysplastic syndrome

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Objective
Copy number analysis on a patient initially diagnosed with B-ALL, who relapsed and eventually progressed to treatment-related myelodysplasia (t-MDS), revealed the aberration of miRNAs. Profiling of miRNA expression was conducted in sixteen additional patients and five healthy donors in order to gain insight in the molecular biology of childhood MDS.

Methods
DNA and RNA of bone marrow (BM) mononuclear cells were isolated using Qiagen kits, ensuring the preservation of miRNAs. ArrayCGH was performed using a 180K custom designed Agilent platform. Profiling of 755 miRNAs was done using a previously described high throughput qRT-PCR platform.

Results
ArrayCGH analysis on the t-MDS sample of a child initially diagnosed with B-ALL revealed a 26.8Mb deletion on chromosome 6 encompassing 159 coding genes and miR-548a-1. Unsupervised clustering of miRNA profiles of the different disease stages of this patient (diagnosis, relapse, t-MDS and remission) showed differential expression of multiple miRNAs. This finding prompted us to investigate sixteen additional childhood MDS patients.
Six miRNAs were found to be significantly differentially expressed between childhood MDS patients and healthy BM donors: miR-618, miR-34b, miR-223, miR-145*, miR-93* and miR-197; with miR-618 most significantly downregulated in MDS (p= 0.0019, Benjamini Hochberg corrected). Correlation analyses with mRNA data identified several strongly anti-correlated protein coding genes, which are now under further investigation.
No significant differences in miRNA expression were found between refractory cytopenia of childhood (RCC) and high grade MDS (RAEB(t)) in this small cohort. However, some miRNAs are borderline and might hint towards interesting biological differences. One of them is miR-196b, lying within the HOXA gene cluster previously linked to childhood MDS.

Conclusion
In this study, we showed the deregulation of miRNA expression in childhood MDS even in the absence of gross chromosomal re-arrangements. Indeed, more than half of the patients have a normal karyotype. The study of the miRNome of childhood MDS is therefore promising to unravel pathogenetic mechanisms and to identify new therapeutic targets.

P.32 The generation and activation of Myeloid-Derived Suppressor Cells in Multiple Myeloma

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Multiple Myeloma (MM) is an incurable malignancy of terminally differentiated plasma cells, which are predominantly localised in the bone marrow. The presence of an immunosuppressive bone marrow microenvironment and dysfunction of immune cells are both described in MM patients; however the mechanisms controlling this immunosuppression are not well defined. Recently, a heterogeneous population of immature myeloid cells, the so called myeloid-derived suppressor cells (MDSCs), were identified to be present and active in MM *(Van Valckenborgh et al, Leukaemia 2012, April 23)*. MDSCs are thought to promote cancer progression by their T-cell suppressive capacity, however in MM little is known about the generation and activation of these MDSCs.
In this study we investigated the effects of the myeloma micro-environment on the total MDSC population using the 5T33MM mouse model. In a first instance, MDSCs (CD11b+) were isolated from the bone marrow of nave C57BL/KaLwRij mice and cultured in conditioned medium (CM) derived from 5T33MMvt cells. Increased viability and reduced apoptosis of MDSCs cultured in MM CM compared to control medium was observed. In literature GM-CSF is described as a major survival factor for MDSCs. The pro-survival effect induced by the MM CM could be abrogated by the use of a GM-CSF antibody. By Western Blot we also determined an increase in anti-apoptotic factors Mcl-1 and Bcl-xL, and an activation of the STAT3 pathway in the presence of CM. Furthermore, we cultured CD11b- cells in CM of 5T33MMvt cells up to 6 days and found a significant increase in the number of CD11b+ cells, indicating that MDSCs are generated in MM conditions. Importantly, MDSCs cultured or generated in MM CM have an increased T-cell suppressive capacity compared to control medium.
In conclusion, these data reveal MDSCs can be generated and activated in MM conditions *in vitro* by the presence of essential cytokines, including GM-CSF. Experiments on human material are currently performed to better understand the role and mechanisms of MDSCs in MM pathogenesis.

P.33 Hematological and molecular responses in a case of refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) treated by Lenalidomide

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RARS-T is a rare entity and is defined as an overlap syndrome with features of both myelodysplastic syndromes and BCR-ABL-negative myeloproliferative neoplasm, including marked thrombocytosis associated with abnormal megakaryocytes.Recently, lenalidomide treatment resulted in hematological responses in 2 patients with RARS-T. We report a third case of RARS-T that was successfully treated by lenalidomide.
A 84-year-old woman consulted for unexplained normocytic anemia (Hg 7.7 g/dL) with marked thrombocytosis (1.515x10^9/L). Peripheral blood smear showed a left shift in neutrophils and Bone marrow cytology disclosed hypercellularity with presence of ringed sideroblasts who constituted 90% of erythroid precursors. Erythropoiesis was hyperplasic, with evidence of dyserythropoiesis. The granulocytic series showed signs of dysgranulopoiesis. The most prominent feature was the presence of atypical megakaryocytes of different sizes and shapes with hypolobated nuclei. Metaphase cytogenetics revealed the presence of 5q- in one mitosis, which could not be confirmed by FISH using a specific EGR1(5q31) probe. The presence of the JAK2-V617F mutation was estimated in 12.5-31% of JAK2 alleles. Based on these findings, RARS-T with a JAK2-V617F mutation was diagnosed. After transfusion of 4 units of red blood cells, she was started on lenalidomide 10 mg daily. Her platelet counts dropped 281x10^9/L, leukocytes normalized and she became transfusion-independent after four months of treatment. After ten months, atypical megakaryocytes and ringed sideroblasts had disappeared in new bone marrow cytology and the PCR for the JAK2-V617F mutation became negative. Lenalidomide treatment was continued, but later stopped after two years because of asthenia. During the active treatment period, the patient received only one RBC transfusion. The patient is currently followed without active treatment with normal platelet and leucocyte counts and a stable macrocytic anemia (Hg of 9.4 g/dL).
Some authors suggest to discard the RARS-T category since it is a rare disease with features and treatment options similar to ET. The described cases of RARS-T patients that were successfully treated with Lenalidomide suggest a distinct pathophysiology and a different therapeutic strategy for these patients

P.34 Multiple Myeloma in the era of novel agents: a single center experience

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Introduction
The positive impact of the novel agents on the prognosis of multiple myeloma has been demonstrated in several phase III clinical trials. However, clinical trials may not reflect the real life situation because of selection bias.

Study aim
Our aim was to study overall survival in a consecutive cohort of patients exposed to novel agents.

Patient population and methods
A total of 63 consecutive symptomatic myeloma patients diagnosed and treated in the department of clinical hematology in UZ Brussel between January 1st 2007 and December 31st 2011, were included. Of these, 59 received at least one line of anti-myeloma treatment. Patient characteristics: M/F (46%/54%); ISS stage I (47%), II (31%) and III (22%); median age at diagnosis: 66 years; creatinine clearance <60ml/min in 39%. Kaplan-Meier method was used for survival analysis, also performed separately for patients eligible or not for autologous stem cell transplantation (ASCT).

Results
First-line treatment was thalidomide-based in 44%, bortezomib-based in 46% and lenalidomide-based in 5% of cases. Only 19 (33%) patients were included in clinical trials. With a median follow-up time of 24 months, the median overall survival (OS) time for the total group (n=63) was 41 months with a 3-year estimated OS probability of 60%. The mean OS of the 59 treated patients was 48.2 (40.2 - 56.1) months with a 3-year OS probability of 64%. The mean OS of treated patients stratified according to ISS stage was 51.1, 40.6 and 23.8 months for ISS groups I, II and III, respectively (I *vs.* III, $p<0.05$). Median OS was only reached in the non-transplant group: 38.6 (31.6 - 45.6) months. The 3-year OS probability for the transplant group was 86 13%.

Conclusion
Our data confirm the positive impact of novel agents on OS of myeloma patients in a real-life single centre setting. The impact is more apparent for patients eligible for ASCT. The prognostic role of the ISS staging system is maintained in the era of the novel agents.

P.35 Performance evaluation of two automated digital morphology systems, Cellavision and HemaCAM in comparison with classical microscopic analysis focusing on rare events counting and on the pre-classification efficiency of the automated systems

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Introduction

Differentials on blood smearing for rare events show a well-known high CV in classical optical microscopy (CM), due to the small number of cells analysed and the subjectivity of classification without possibility of cells review. Digital microscopes give pre-classification of WBCs and permit easy abnormal cells recognition.

Material and methods

We compare three techniques of microscopy, the CM and two digital microscopes, CellaVision (Sysmex) and HemaCAM (Horn Imaging), in term of sensitivity to detect rare cells and of reproducibility of counting. We measure the efficiency of the pre-classification of the six subtypes of cells (neutrophils, eosinophils, basophils, lymphocytes, monocytes and NRBC) by the two digital microscopes.

Patients (n=48) from routine samples were selected for their positivity for IG and/or NRBC and/or blasts. For each patient, ten smears slides were done and analysed by the three techniques.

Results

The CellaVision demonstrates the highest sensitivity for detection for the three types of abnormal cells.

For NRBC, a significant difference exists between the three techniques with a correlation superior to 0.87 between each pairs of microscopy. The higher mean of NRBC is attempted with CellaVision and the lower CV by CM.

For IG and blasts, there is no significant difference between the three techniques with a correlation superior to 0.75 and 0.89 respectively. However, CVs of the two digital microscopes are obviously lower than those of CM.

For the study of the efficiency of pre-classification, there is a significant difference between the number of cells needed to be reclassified between CellaVision and HemaCAM, the median being of 6 and 8.5 respectively.

Conclusion

The CellaVision demonstrates the highest sensitivity for all rare events and the lowest CV for IG and blasts counts. Furthermore, the CellaVision performs a better pre-classification permitting a greater time-saving.

P.36 Management of fit older AML: major prognostic value of cytogenetic markers is confirmed whatever the age

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Introduction

Median age of AML at diagnosis is 67 years. Major problems remain frailty and biological features increasing resistance to chemotherapy. However, some patients are cured: we study impact of prognostic factors (PF) in order to identify patients deserving intensive treatment.

Population and methods

We retrospectively reviewed 77 patients over 60 yo between 1997 and 2011. Fit patients (PS<2, irreversible comorbidities < 3 and no geriatric syndromes) received intensive chemotherapy. Several factors were assessed in a multivariable analysis, in order to define their prognostic significance according to age subgroups. Kaplan-Meier curves were used to compare PF.

Results

50% of all patients reach a complete remission post induction. Overall survival (OS) for patients over 60 yo is 48%; 31% and 17% at one, two and five years, with a median survival of nine months. According to age, OS is better ($p < 0.0001$) for patients below 70 years than above (44% at 2y *vs.* 16%). OS in patients treated with curative intent is better than supportive care (62% at 1y *vs.* 6%, $p < 0,0001$). Karyotype was unfavourable in 65%, intermediate in 21% and favourable in 10%. This last group provides a better prognosis whatever the age (OS at 2y 53% *vs.* 20%, $p = 0.007$). Taking into account cytogenetic data and age, median survival of patients over 70 with favourable karyotype was very similar to population below 70 (38 and 44% at 2y). Death due to uncontrolled disease (51n, 66%) remains a major concern compared to death induced by treatment related toxicity (9n, 15%).

Conclusion

Cytogenetic data is the PF with the strongest predictive positive value in term of outcome whatever the age. For patients above 70, individual assessment should be refined in prospective studies to improve therapeutic decision making. The survival of elderly AML patients remains poor, more because of disease resistance than treatment toxicity.

P.37 Incidental diagnosis of Fanconi anemia in an adult patient with myelodysplastic syndrome

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A 35-year old woman was diagnosed with a myelodysplastic syndrome. She presented with an oral squamous cell carcinoma at the age of 30 years and at 34, a melanoma was noted. Because of the occurrence of multiple malignancies at a relatively young age, a Li-Fraumeni syndrome was suspected. Mutation analysis of the *TP53* gene was however negative. Array-CGH analysis of a peripheral blood sample of the patient showed a trisomy 1q, gain of 3q, monosomy 7 and an interstitial deletion of 16q24.3. This deletion comprised an intragenic deletion (around 15 kb and at least exons 11-15) of the *FANCA* gene. These results were reminiscent of the karyotypic abnormalities in the myelodysplastic bone marrow sample, although the peripheral blood sample showed no blasts. The finding of the above mentioned structural chromosome alterations in a non-malignant peripheral blood sample, together with the presence of an intragenic deletion of the *FANCA* gene is highly suggestive for a diagnosis of Fanconi anemia. In Fanconi anemia, 40% of mutant alleles are large intragenic deletions of the *FANCA* gene. Fanconi anemia is considered as a typical pediatric disorder, as it is usually diagnosed at a median age of seven years and is rarely considered in the differential diagnosis in adult oncology. The diagnosis of Fanconi anemia is further strengthened by the history of an oral squamous cell carcinoma in our patient, since the relative risk of Fanconi anemia patients is high for the development of oral cancers. As Fanconi anemia patients show a

hypersensitivity to DNA damaging drugs, an *in vitro* chromosomal breakage blood test will be performed to confirm the diagnosis of Fanconi anemia. In addition, mutation analysis of the other *FANCA* allele is planned. The confirmation of the diagnosis is important in the light of family counseling, since Fanconi anemia is autosomal recessive and also for the treatment of the different malignancies, since due to the hypersensitivity to some chemotherapeutic agents, major adverse events need to be considered.

P.38 Large pericardial effusion after heterologuous stem cell transplant : about two cases

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Pericardial effusion is a rare complication of HSCT. We report about two adult patients who developed constrictive pericarditis after HSCT.
The first patient, a 63-year old man developed dyspnea and hypotension four months after non myeloablative HSCT for refractory mantle cell lymphoma. Cardiac US displayed a large constrictive effusion. Pericardial window was performed revealing an exsudative liquid. Bacterial and viral infectious causes, as well as localized relapse could be ruled out. Pericardial effusion did not reappear eight months after treatment.
The second patient, a 31-year old woman developed chest pain and gradual dyspnea six months after HSCT for acute myeloid leukaemia. She was currently treated for acute hepatic GVHD using high dose corticosteroids and mycophenolate mofetyl. Cardiac US showed a circumferential pericardial effusion with compression of the right ventricle. She was effectively treated with pericardial window and remains asymptomatic seventeen months after fenestration.
Cardiac tamponade is a rare complication of post allogenic transplant. A review of the literature discusses the various causes of pericardial effusion. If localized relapses and infectious serositis must be excluded, isolated serositis may be a feature of chronic graft versus host disease. Rapid surgical intervention is required in cases of right ventricle compression and is potentially curative.

P.39 Simultaneous diagnosis of CLL and CML in a single patient with evidence for two different cell clones

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We report the case of an asymptomatic 75-year old man who presented with a high white blood cell count (85 x 10^9/L) and slight thrombocythemia (455 x 10^9/L) without anemia. The microscopic formula revealed neutrophil and lymphocytic percentages within ranges of normal values, the presence of some young components (myelocyte: 1%, metamyelocyte: 5.5%, myeloblasts: 1%) and also the presence of Gumprecht shadows in lymphocytes. Other blood tests were unremarkable except for Lactate DesHydrogenase (1,031 IU/L) above ULN. Immunophenotyping of peripheral blood showed a chronic lymphocytic leukaemia (CLL) immunophenotypic pattern (CD19+, CD5+, CD23+, ? light chain at low density). Cytogenetic analyses, performed on peripheral blood, revealed two different clones with the presence of a Philadelphia chromosome (t(9;22)(q34;q11)) in 41% of the cells but also deletion of 13q14 (46% of the cells) and 11q22 (21% of the cells) loci. Bone marrow biopsy confirmed the coexistence of chronic myeloid leukaemia (CML) and CLL, while molecular analyzes demonstrated the presence of both BCR-ABL rearrangement and monoclonality for the loci I, II and III of the Heavy chain of the Immunoglobin gene. In order to determine whether the two diseases originated from the same clone or not, CLL cells (defined as CD19+CD5+ cells) and granulocytes (isolated based on the FSC / SSC pattern) were isolated by flow cytometry. While deletions of loci 13q14 and 11q22 were present in all CLL cells but absent in all granulocytes, the Philadelphia chromosome was demonstrated in all granulocytes but in no CLL cells. Based on these data, we conclude that the patient presented with two different diseases originating from two different cell clones. The patient, first treated with hydroxyurea (for 10 weeks) and later with imatinib (for nine weeks thus far), experienced a rapid hematologic remission of the CML component, but with persistence of lymphocytes with Gumprecht shadow. A similar case report has been recently reported (D'Arena *et al.*, JCO, Vol 30, 2012) except that, here, CML occurred seven years after CLL diagnosis.

P.40 Hyperhaploid plasma cell myeloma: a rare case report

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Multiple myeloma is an incurable malignancy of plasma cells and is characterized by complex cytogenetic aberrations. The chromosome ploidy number of conventional karyotyping plays a pivotal role in the risk stratification of newly diagnosed myeloma . There are four distinct groups: Hyperdiploid , Pseudodiploid, Hypodiploid and Near-tetraploid. The **hypodiploidy** (or hyperhaploidy) is a rare event in myeloma with only six cases published to date. The prognoses appear to be poor.
In this case report, we present a further case with hyperhaploid karyotype (31,X,+3,+5,+7,+9,+11, t(3;14)(q21~25;q24), +15,+18,+19 [5] / 46,XX [16]). As is the case for this myeloma, the extra chromosomes present in these cases involved the odd numbered chromosomes. We have correlated results of the conventional karyotype, Fluorescence *in situ* hybridization (FISH) and Comparative Genomic Hybridization (CGH). FISH and CGH were performed on plasma cells after CD138 positive separation. FISH result confirmed the ploidy of the karyotype with monosomy of the chromosomes 4;13;14;16 and 17. CGH profile (Agilent technology) was consistent with **a typical hyperdiploid** cell line! The ploidy of this clone could not have been determined based a -array analyse alone. Use a *SNP/CNV* array would be useful to identify such cases.

P.41 Central nervous system relapse of chronic myeloid leukaemia after allogeneic hematopoietic stem cell transplantation effectively treated with dasatinib

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Central nervous system (CNS) relapse of chronic myeloid leukaemia (CML) after allogeneic haematopoietic stem cell transplantation (allo-SCT) is rare and anecdotal. Dasatinib has been showed to cross the blood-brain barrier, and might be therefore a good candidate as salvage therapy in this particular setting.

A 25 year-old male has been diagnosed with CML in accelerated phase in September 2009. He received a related allo-SCT after a myeloablative conditioning in March 2010 after a combined therapy with nilotinib and chemotherapy. BCR-ABL transcript was undetectable on day +30 but was back to positive on day +100. He received a donor lymphocyte infusion along with nilotinib and achieved a complete molecular remission, lasting for 18 months. He subsequently developed an extensive chronic graft-versus-host disease (cGVHD) in February 2011 and the immunosuppressive therapy was resumed. In December 2011, he developed lower limbs paraparesis and back pain. Magnetic resonance imagery was unremarkable. Cerebrospinal fluid (CSF) analysis showed increased cell count with CML features. BCR-ABL amplification test on the CSF was technically not feasible. However, a mutation of the E255K domain, which is known to be resistant to nilotinib, was identified on the bone marrow. Intrathecal administration of cytarabine, methotrexate and hydrocortisone was initiated until CSF clearance along with oral dasatinib. We subsequently observed a slow but significant neurological recovery. BCR-ABL remained undetectable thereafter.
Here, we report on a CNS relapse of CML after allo-SCT in a patient on nilotinib. This relapse was effectively salvaged with intrathecal chemotherapy and oral dasatinib. Our observation confirms previous reports on the ability of dasatinib to cross the blood-brain barrier. Dasatinib should be therefore considered in the setting of CNS involvement of CML but further trials are warranted.

P.42 Microvesicles bearing Tissue-Factor: a new potential biomarker for thrombosis in acute promyelocytic leukaemia

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Introduction
Patients with hematological malignancy have a 28-fold increased risk of venous thromboembolism (VTE). Among patients with acute myelogenous leukaemia (AML), the 2-year cumulative incidence of VTE is 5.2%. The induction mechanism of a hypercoagulable state is not fully understood. Multifactorial aspects such as patients' immobility, chemotherapy adverse effects or the overexpression of several procoagulant substances (i.e. tissue factor (TF)) by cancer cells are often evoked. Several studies strongly suggest that microvesicles (MVs) harboring TF may have a procoagulant role in promoting deep vein thrombosis and possibly disseminated intravascular coagulation (DIC) commonly seen in acute promyelocytic leukaemia (APL)

Objectives
The aim of this study is to assess the capacity of untreated (APL) cells to shed procoagulant MVs.

Methods
APL cell lines (NB4 and HL-60) were cultured for 48h in liquid medium at 600,000 cells/mL. Cells and MVs were separated by filtrations (Millipore 0.1-0.22-0.45-0.65m). The Pro-Coagulant Activity (PCA) was assessed by thrombin generation assay. Alternatively, MVs were incubated with anti-TF antibodies (10g/mL), with annexin V (0,5M) to assess the contribution of TF and phospholipids to the PCA. Alternatively, the cells were incubated with with $HgCl_2$ (an activator of TF).

Results and discussion
NB4 cells have a high PCA mainly triggered by MVs of size under 0.45 m. Thus, NB4 cells spontaneously release MVs of various size, which can augment TGA. By using an anti-TF antibody (HTF-1) and annexin V, we confirm that the PCA of MVs is related to the expression of active TF and PL. Interestingly, we show that HL-60 cells have a weaker PCA since TF is mostly present in an inactive form. Moreover HL-60, do not produce MVs<0.65 m associated with PCA.

Conclusions
Microvesicles could have a predicting value for venous thromboembolism and DIC in patients with acute promyelocytic leukaemia and could inform haematologists for the thrombosis prophylaxis.

P.43 Pneumatosis intestinalis as a sign of acute graft versus host disease. Pneumatosis intestinalis as a sign of acute graft versus host disease

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A 30-year-old man was referred for mild abdominal pain and diarrhea. He had a history of obesity treated by an intestinal bypass. Five weeks earlier, he had undergone an allogeneic stem cell transplantation from an unrelated matched donor after a myeloablative regimen containing cyclophosphamide and total body irradiation, for aPhiladelphiapositive acute lymphoblastic leukaemia. Graft versus host disease (GVHD) prophylaxis consisted of a combination of anti-thymocyte globulins, cyclosporine and methotrexate.
Physical examination showed a poor performance status, with a diffuse fluid retention and a sensitive abdomen. Blood tests were in the normal range except for a grade 3 anemia (Hb 7.6g/dl) and infra-therapeutic cyclosporine-A levels. BCR-ABL fusion transcripatients were not detected. Upper digestive tract endoscopy identified multiple bleeding ulcers with an underlying histologically proven acute GVHD.
Abdominal CTrevealed the presence of intramural gas in the wall of the whole colon, with numerous air bubbles around the bowel (pneumoperitoneum). Bacteriological and virologic evaluation of the stools failed to identify any infection. The patient was managed conservatively with large spectrum antibiotics, parenteral nutrition, and prednisone at a dose of 1 mg/kg/day. Immunosuppression was modified for etanercept and tacrolimus, with rapid digestive improvement.
This report deserves attention because (i) diffuse intestinal pneumatosis represents an unusual manifestation of acute GVHD, that can be successfully treated with a conservative approach, (ii) gastrointestinal by-pass can be associated with a poor cyclosporine-A reabsorption that can further be responsible for the occurrence of acute GVHD

P.44 Localised leukaemic cerebral relapse following zygomycosis of the ear after chemotherapy consolidation for AML M1

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A 63-year-old male diagnosed with AML M1 associated with EVI gene rearrangement developed, during induction chemotherapy, a left external otitis related to a mucormycosis, that required antifungal therapy with liposomal amphotericin B. Consolidation therapy was started under antifungal therapy; on day 32, the

patient complained of headache, and rapidly developed confusion and obnubilation; CSF analysis was normal but cerebral MRI revealed an osteolysis of the left tympanal bone compatible with a malignant otitis externa (MOE). Further therapy included surgery as well as prolonged administration of posaconasole. Matched unrelated donor reduced-intensity chemotherapy allogeneic stem cell transplantation (MUD RIC-SCT) was performed under posaconasole, without any further infectious complications. The patient achieved a complete remission that last for three years, despite a chronic GVHD successfully managed with mycophenolate mofetil and methylprednisone. However, he was admitted for a brutal deterioration of conscienceness, that was proven to be related to a localized relapse of his leukaemia, at the site of the previous MOE. No further treatment was proposed.
We here report an unusual site of cerebral relapse after a malignant otitis externa related to zycomycosis, in a patient with prolonged survival after MUD RIC-SCT for EVI-1 AML.

P.45 Cerebrospinal fluid invasion in an APL patient. A case report

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Leukaemic meningitis in APL patients is a rare occurence and treatment of blastic meningitis is based on experience with ALL patients. We report the case of a 58 year old patient who was admitted for acute promyelocytic leukaemia. After classical induction therapy with Aracytine, Daunorubicine and all transretinoic acid, the patient developed a marked increase in bone marrow and circulating blasts. A salvage therapy with high-dose Cytarabine, Mitoxantrone and arsenic trioxyde was complicated with differentiation syndrome.
Concurrently, the patient developed hearing loss dizziness and vertigo. Cerebral MR showed meningeal thickening and perineural infiltration of the facial nerve. Lumbar punction confirmed the presence of promyelocytes in the cerebrospinal fluid, thus confirming the diagnosis of blastic meningitis. The patient was treated with serial intrathecal injections of Methotrexate which allowed rapid recovery from the neurological symtoms except hearing loss. The patient achieved complete haematological and cytological response. During anthracycline consolidation based on the PETHEMA regimen adding all trans retinoic acid, the patient showed isolated cerebral reappearance of promyelocytes. She therefore benefitted from a new series of intrethecal injections and whole brain radiotherapy.
The patient remains in complete response two years after initiation of treatment.

P.46 Purple skin lesions and low back pain

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Systemic mastocytosis (SM) is a clonal disorder of the hematopoietic system characterized by an abnormal accumulation of mast cells in several tissues. Mast cell infiltration can be responsible for organomegaly, malabsorption, osteolysis with pathological fractures or bone failure.
A 53-year-old male, without any relevant medical history, was referred for low back pain lasting for 5 weeks. He presented with a dark brown maculopapular rash for several years. Computed tomography showed multiple vertebral collapses with diffuse osteopenia. Magnetic resonance imaging (MRI) of the spine demonstrated a hypercellular marrow suggesting an haematological disease. Blood tests were in the normal range. Bone marrow trephine identified multifocal clusters of spindle-shaped mast cells, expressing tryptase, CD117 and CD5. Serum tryptase was slightly elevated. Cytogenetic analysis was normal and molecular biology failed to identify any c-kit mutation.
After six months of treatment with H1 and H2 blockers, biphosphonates and imatinib, the patient is asymptomatic, with improvement in bone mineral density and bone turnover markers. SM is usually an indolent disease. Here we describe an unusual presentation diagnosed in a context of acute low back pain.

Abstracts posters platelets & coagulation (P.47-P.50)

P.47 Impact of the oral and direct Factor Xa inhibitor Rivaroxaban on five routine coagulation assays, an in-vitro and ex-vivo study

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Objectives
The oral direct factor Xa (FXa) inhibitor Rivaroxaban has been developed for treatment and prophylaxis of thromboembolic disorders. The conventional coagulation tests can be affected by the Rivaroxaban. The purpose of this study is to evaluate the influence of Rivaroxaban on most common routine coagulations assays in order to correctly interpret the test results.

Methods
Rivaroxaban was spiked at increasing concentrations into normal human PPP from 5 healthy volunteers. Two Prothrombin Times (PT) (Quick PT - STA NEOPLASTIN R, Roche/Stago and Owren PT (Owren PT, Medirox), activated partial thromboplastin time (aPTT - PTT-A, Stago and Actin FS, Siemens), thrombin time (TT - Thrombin10, Stago), antithrombin (Coamatic AT anti-Xa, Chromogenix) and fibrinogen (STA-Fibrinogen, Stago) were measured. Tests were performed on a STA-R (Stago) and responsiveness to Rivaroxaban was assessed. PT and aPTT were also evaluated on selected Rivaroxaban treated patients and correlated with their Rivaroxaban concentrations (Liquid anti-Xa Stago).

Results
Rivoroxaban prolonged Quick and Owren PT in a concentration-dependent way with inter-variability increased at high concentrations levels. The Quick PT assay was more sensitive compared than the Owren PT assay. In addition, Neo-R demonstrated a high sensitivity to Rivaroxaban. aPTT was also prolonged in a concentration-dependent manner but this test seemed less sensitive than PT. Similar results were observed on *ex-vivo* samples but individual variability did not allow the use of PT to estimate Rivaroxaban concentration. The antithrombin assays showed an overestimation of the AT concentration with the Xa-based assay. Rivaroxaban did not interfere with fibrinogen assay.

Conclusions
In these assays, Rivaroxaban affects all conventional clotting tests. These interferences should be known to ensure appropriate interpretation of results. Despite a high sensitive reagent to Rivaroxaban, the individual variability does not allow an estimation of the anticoagulant concentration. Therefore, Anti-Xa activity

seems to be required for this purpose.

P.49 Impact of thromboplastin's choice on VKA treated patients monitoring

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Some data could evoke an INR overestimation by the reagent STA-Noplastine R (Neo R) (Stago).
The aims of our study were 1) to estimate the potential biais of INR between fresh and frozen samples and 2) to compare the different reagents and systems on the market.
Neo R and STA-Noplastine CI+ (Neo CI+) on STA-R (Stago), Recombiplastin2G (R2G) on ACL Elite (Instrumentation Laboratory) and Innovin on BCS (Siemens) were calibrated and tested following manufacturer's recommendation. For R2G, both factory ISI and calibrated ISI (ISI cal reagent) were tested. The Neo R ISI is determined relative to the fourth WHO international standard (rTF/09). The impact of ISI variation of Neo R on INR results has been estimated with two lots (ISI 0.98 and 1.07). Selected samples from untreated and AVK treated patients were tested within four hours after blood sampling and after freezing (six month at -70C).
The evaluation of plasmas freezing effect on INR, performed on Neo R and Neo CI+, demonstrates opposite reactions, respectively -9% and +8% between fresh and frozen samples.
Comparisons between reagent's laboratory (Neo R, ISI =0.98) and the different reagents are:
(1) Recombiplastin 2G, factory ISI Y= 0.939 X + 0.071, r=0.991
(2) Recombiplastin 2G, calibrated ISI Y= 1.081 X - 0.186, r=0.986
(3) Innovin Y=0.965 X + 0.122, r=0.977
(4) STA-Noplastine CI+ Y=1.109 X - 0.110, r=0.945
Comparison between the 2 lots of Neo R demonstrates a perfect match between the 2 lots (Y= 1.038 X + 0.020, r=0.989).
The ISI difference between the two lots of Neo R and the differences observed between the various reagents are without clinical impact in monitoring VKA patients. The differences between fresh and frozen samples and the type of each reagent may explain some differences observed during this study. For Neo R versus Neo CI+ comparison, the impact of freezing and thawing is weak but opposite and this emphasizes the importance of working on fresh samples in the process of determining and validating the calibrations.

P.50 Rituximab as a maintenance treatment in thrombotic thrombocytopenic purpura : a case report

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Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease leading to multiple organ failure. Diagnosis is based on the presence of thrombocytopenia, and mechanical hemolytic anemia due to microangipathy. Renal impairment and central nervous system involvement may result from organ damage. It is now recognized that auto-immune TPP is the consequence of reduction of blood levels of the disintegrin and metalloprotease with thrombospondin motifs (ADAMTS)-13 by an anti ADAMTS 13 antibody. Rituximab, an anti CD20 antibody has been largely described in the salvage treatment of refractory TTP. Some data also report an efficacy in the prevention of TTP recurrence.
We report the case of a 41-year-old women hospitalised in our unit for recurrent TTP, appearing in a viral or bacterial context. At diagnosis, the patient displayed severe deficiency in ADAMTS13 activity and high level of Anti-ADAMS 13 antibodies between 2005 and 2011. Although plasma exchange allowed a good clinical answer, anti ADANTS 13 antibody titer remained high between two episodes. At the last relapse we decided to start maintenance treatment with Rituximab. One year after treatment, the patient displayed a subnormal ADAMTS 13 activity and a reduction of anti ADAMTS 13 antibody titer.
No TTP recurrence was described despite several viral infection and one episode of bacterial skin infection.

Abstract posters red blood cells and transfusion (P.51-P.53)

P.51 Relationship between thrombin generation and blood flow velocity in large cerebral arteries of children with sickle cell disease

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Introduction
Sickle cell disease (SCD) Children have increased risk to develop stroke. High risk children are screened using Transcranial Doppler ultrasonography (TCD) which measures blood flow velocity in large cerebral arteries. We recently showed that thrombin generation (TG) was increased in some SCD children. However, the link between TG and the risk of stroke in SCD is still unknown.

Objective
To assess the relationship between TG and the time-averaged mean of the maximum velocity (TAMMV) measured using TCD in the cerebral arteries of SCD children.

Methods
TG and TCD were performed in 61 SCD children (2-16 years old: median: 8) at steady-state. TG was triggered in the platelet-poor plasma using 1 pM tissue factor and 4 M phospholipid with thrombomodulin. To normalize TG for age, ratios of endogenous thrombin potential (rETP) and peak height (rPeak) were calculated as patient's value divided by the mean value of controls of the same age range previously determined in our laboratory. We considered the highest TAMMV value in both sides of the internal carotid, medium and anterior cerebral arteries. LDH, total hemoglobin and reticulocyte count were also measured as markers of hemolysis. Correlations were assessed using the Spearman's test. $p < 0.05$ was considered significant.

Results
Overall, reliable velocity measurements were obtained in 54 patients. Median (IQR) values for these patients were: TAMMV: 116 (104.8 - 131.3) cm/s; rETP: 1.55 (1.12 - 1.86) and rPeak: 1.96 (1.16 - 2.31). TAMMV correlated positively with both rETP and rPeak. TAMMV, rETP and rPeak correlated negatively with age and total hemoglobin, and positively with LDH. Reticulocyte count correlated with rETP and rPeak.

Conclusion
TAMMV and TG seem to have similar age distribution in SCD children. They also correlate similarly with markers of hemolysis.

This further supports the hypothesis that hemolysis could be the mechanism underlying the pathophysiology of both vasculopathy and hypercoagulability in SCD. This study also suggests the possible contribution of abnormalities of coagulation in the onset of cerebrovascular disease in SCD children.

P.52 Newborn screening for Sickle Cell Disease in Brussels, a program with an ongoing clinical outcome improvement

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The aim of our study, conducted in Brussels Region, was to assess whether there is an ongoing improvement of clinical outcome of children with sickle cell disease (SCD) detected by the newborn screening program.
To evaluate the improvement in comprehensive care, we reviewed data of children born from January 1st 2000 to December 31st 2003 (group A) and from January 1st 2005 to December 31st 2008 (group B). All data were recorded from January 1st 2000 to December 31st 2005 for group A and from January 1st 2005 to December 31st 2010 for group B. Both groups had the same follow-up period accounting for 118 patient-years in group A and 259 patient-years in group B. Median follow-up was 3.5 yrs (range 2.06-5.83 yrs) and 4.1 yrs (range 2.08-5.96 yrs) in group A and B respectively. The SCD related events, the hospitalized days and several biological parameters were reviewed and compared during the study follow-up between the two groups.
Among the 98 patients identified with SCD at birth, 33 (16 girls and 17 boys) and 65 (37 girls and 28 boys) belonged to group A and B, respectively. In group A, 25 children were HbSS, 2 HbS^+ and 6 HbSC. In group B, 53 were HbSS,5 HbS , 5 HbS^+ and 2 had an other genotype. The proportion of patients having presented severe anemia and acute chest syndrome was significantly lower in group B than in group A. No difference was observed between both groups for dactylitis, VOC, septicemia and clinical neurological event. No patient died during the study period. Haematological parameters at one year of age were not different between both groups.
In conclusion, newborn screening is obviously recognised as a precious tool to identify patients with SCD. Our results demonstrated that its sustained effectiveness is really and clearly proven when it is coupled with a comprehensive and dedicated treatment program including close and regular parent education.

P.52 Neuropeptides to replace serum in cryopreservation of mesenchymal stem cells?

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Background
The therapeutic potential of human mesenchymal stem cells (MSC) has generated considerable interest in a wide variety of areas. Allogeneic MSC banking is feasible but the optimal technique of cryopreservation remains to be determined.

Methods
In this study, to increase cell survival and proliferation rates after thawing and to eliminate the need for FBS, neuropeptides of the VIP/GIP/PACAP family were added in the cryo-medium. Cell survival was analysed by a Trypan blue dye exclusion assay. Cell proliferation of cryopreserved MSC was determined after 7 days of culture. Fourteen different conditions were tested.

Results
The addition of neuropeptides in the cryo-medium always produced better proliferation rates and consequently a better cellular output. Without FBS, the best cellular output was achieved with DMEM + 10% DMSO + 1M PACAP as cryo-medium (4.10.9 fold increase). With FBS, the best cellular output was obtained when MSC were cryopreserved in DMEM + 5% DMSO + 30 mM trehalose + 10% FBS + 1 M GIP (5.21.4 fold increase).

Conclusions
FBS could be replaced by VIP, GIP or PACAP without loss in cell survival and proliferation potential. Moreover, the addition of VIP, GIP or PACAP in the cryo-medium containing FBS could increase cell proliferation rate and consequently cellular output.

P.53 Acute liver failure in a patient with sickle/beta-thalassemia (S/β)

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A 42-year-old greek male with a compound heterozygous haemoglobinopathy, i.e. sickle/beta-thalassemia (S/) was referred for jaundice and fatigue. He reported several vaso-occlusive crises during the past year, and a history of biliary lithiasis in the context of chronic hemolysis. Physical examination revealed mild hepatomegaly with splenomegaly. Work-up failed to identify any obstructive nor infectious etiology. He rapidly developed clinical and biological signs of liver failure, leading to an emergency orthotopic liver transplantation Exsanguinotransfusion performed before the surgical procedure allowed to lower the HbS level from 81 to 8%. Histology of the native liver showed massive congestion of the sinusoids with hepatocytic necrosis and cholestasis. On day 7 post-transplantation, despite a low level of HbS, the patient developed a stenosis of the celiac artery leading to loss of the graft, and was retransplanted. Histology of the transplanted liver showed multiple ischemic lesions with persistance of numerous sickle cells. The patient further developed a gram negative septicemia leading to multiple organ failure and to death.
This reports illustrates that, despite many patients with s/beta+ thalassemia have milder symptoms than those with homozygous sickle cell disease, severe ischemic lesions leading to acute liver failure can be encountered. The outcome of liver failure is usually fatal.

Abstract posters stem cell biology and transplantation (P.54-P.63)

P.54 Establishment of transgenic human embryonic stem cell lines for the study of human hematopoiesis

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Introduction
The derivation of human embryonic stem cells (hESC) has opened the path for the study of human development. During the last decade, the number of murine transgenic models has vastly increased, resulting in developmental insights on a molecular level. In recent years, technical advances have allowed us to generate transgenic human embryonic stem cells, by which we can study subsequential stages of development and differentiation *in vitro*.
Key factors during haematopoiesis are cMYB, Runx1 and notch signalling, we thus generated transgenic hESC for the study of these factors. We describe different techniques and models generated for the study of human haematopoiesis, which will provide novel biological insights.

Methods & Results
All transgenic models were made using the WA-01 hESC line (WICELL).
For the cMYBeGFP reporter cell line, a BAC reporter strategy was used. In brief, we targeted the first starting codon of the cMYB gene, coded on the CH17-400L19 BAC plasmid, with an eGFPpA -LoxP-Neo-loxP cassette using recombineering, and nucleofected the targeting construct in single-cell suspended hESC. After transgene validation, the selection cassette was removed.
Since the regulatory elements for runx1 are well described, a simplified reporter construct was made in which eGFP was placed under the control of the runx1 specific enhancer (enh+23) Zinc finger nucleases targeting the human AAVS1 safe harbor site were used (sigma Aldrich). Integration of the E+23eGFP was obtained by homologous recombination.
For cMYB knockdown using shRNAs, a feeder free lentiviral transduction protocol was used. Also for the study of notch signaling during human hematopoiesis, inducible dnMAML1/ICN1 over-expression lines were made using this protocol.
All transgenic lines described above were validated through integration PCR on the gDNA level and FISH, after hESC hematopoietic differentiation culture, reporter expression and transgene effect was validated through flow cytometry and rt-qPCR.

Conclusion
We describe novel models for the study of human hematopoiesis, which will lead to fundamental insights of the function of these genes in both normal development and oncogenesis.

P.55 Expression of the hemogenic transcription factor Runx1 in in vitro generated hematopoietic progenitor cells

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Introduction
During embryonic development, hematopoietic stem and progenitor cells (HPSC) are derived from specialised endothelial cells in the dorsal aorta. Here, hemogenic endothelium cells round up and start budding. The transcription factor Runx1 is essential for a normal budding process since mice embryos deficient for Runx1 die at day 12.5 as a consequence of the absence of hemogenic endothelium and HPSC.
Unlike mice and zebrafish, the mechanisms of the earliest human hematopoiesis remain largely unknown. Human embryonic stem cells(hESC) are a convenient model to study these earliest developmental stages. Using a Runx1 reporter hESC line, we will evaluate the expression and the role of Runx1 during *in vitro* generation of HPSC.

Materials and methods
In order to generate the Runx1 reporter hESC line with eGFP as a marker gene, we introduced a reporter construct based on Bee *et al* (2009) in hESC, using the zinc finger nuclease recombination technology. *In vitro* hematopoiesis was induced by a spin embryoid body(EB) system. We validated reporter capacity using confocal microscopy and flow cytometry.

Results
After induction of differentiation, round haematopoietic cells were microscopically visible starting around day 7. At day 10, the first eGFP$^+$ cells could be detected, which were still present at day 12. Flow cytometric analysis showed 8% of eGFP$^+$ cells on day 7, of which 60% were CD34$^+$CD43$^+$HPC and 40% were CD34$^-$CD43$^+$ more differentiated progenitors. eGFP expression reached a peak of 20% on day 10, yet the fraction of CD34$^+$CD43$^+$ cells decreased while the CD34$^-$CD43$^+$ population increased. The results of the day 12 EBs are in line with day 10, though the total fraction of eGFP$^+$ cells decreased again to 10%. At each time point, eGFP expression was only observed in the haematopoietic populations.

Conclusion
Our findings indicate that the Runx1 reporter hESC line is able to report specific Runx1 expression. This reporter line can be useful to study the expression and role of Runx1 during the *in vitro* generation of HSPC.

P.56 Serum hepcidin following autologous haematopoietic cell transplantation : an illustration of the interplay of erythropoiesis, iron status and inflammation

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Introduction
Hepcidin, the main hormone in iron regulation, has been little studied after HCT.

Patients and methods
Among patients included in our prospective, randomied, trial comparing no erythropoietic therapy, darbepoetin alpha (DA) therapy and DA + IV iron starting on day 28 after autologous HCT, we randomly selected 15 patients in each group. We measured hepcidin levels at various time points as well as their relationships with iron status (serum ferritin and transferrin saturation), erythropoietic activity (sTfR) and inflammation (CRP).

Results
Figure 1 displays hepcidin levels over time in the three groups. We observed a peak in hepcidin levels seven days after HCT, followed by a decrease until day 28 (before DA treatment). Thereafter hepcidin levels in the control group remained stable, whereas those in DA +/- iron groups decreased rapidly until day 60. This decrease was better illustrated when we analysed hepcidin as a percentage of the hepcidin value on day 28 (figure 2). From day 60, hepcidin levels in the DA + iron group increased again to become higher than those in controls on day 100 and those in the DA group on days 100 and 180. We found many significant correlations between hepcidin and ferritin over time, the correlations were stronger when examined in same day samples, especially on days 60 and 100 (r around 0.75). Furthermore, correlations between hepcidin and transferrin saturation values were found on days 60 and 100 (r between 0.39 and 0.65, p values between 0.01 and

<0.0001). On the other hand, we identified negative correlations between hepcidin and sTfR: hepcidin on day 60 correlated with all sTfR levels between day 28 and 100, whereas day-100 hepcidin correlated with sTfR on days 100 and 120. Finally, no significant correlation was observed between hepcidin and CRP, Hb or Hct.

Conclusion
In conclusion, we demonstrated that erythropoetic therapy (DA+/- iron) had an impact on hepcidin, through interaction with erythropoietic activity and iron stores; the effect of inflammation was less apparent.

P.58 Impacts of the JACIE accreditation at the department of Hematology at Jules Bordet Institute (IJB) and the Hemato-Oncology Service of Hopital Universitaire Des Enfants Reine Fabiola (HUDERF).

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Background
In March, 2010, the department of Hematology of the IJB and the Hematology-oncology service of the HUDERF were accredited by JACIE. The objective of this study is to analyse the impacts of the accreditation implementation on the whole activity of these two entities.

Methods
We analyse retrospectively data from the archived documents of the quality system, both before and after March 2010. The documents are evaluated for four domains of impact: change in organisational and quality culture, improvement of professional practice, effects of the quality management on the patients and collaborating centres satisfaction, costs and investments requirements.

Results
1. Major organizational changes with a departitioning and an increased communication between adult and paediatric teams.
2. Improvement of professional practices modified by standardization and large application of regularly updated procedures. Good follow up and documentation of staff training.
3. Patients' satisfaction is well established by their gratitude. Better communication with referring colleagues increasing collaborative work and exchange.
4. Important costs and investments.

Conclusions
Significant impact on daily practice and the whole activity of two services. The implementation of the JACIE project brought dynamism and innovative inputs and was very positive on the quality of the care management despite important investments.

P.59 Protective Isolation in Hematology : Risk Assessment and Environmental Control

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Introduction
Nosocomial Invasive Aspergillosis (NIA) is reported as a major risk for deeply immunosuppressed patients treated for hematological malignancies.
Preparing for JACIE accreditation, we aimed to assess compliance with standards related to Bone Marrow Transplant (BMT) Clinical Unit and minimisation of airborne microbial contamination.

Material and Methods
Six rooms equipped with High Efficiency Particulate Air filtration (HEPA) and Laminar Air Flow (LAF, high-risk unit) and 10 HEPA rooms were controlled respectively twice and once a year by an external organisation, for aeraulic parameters, particle counts (HEPA-LAF and HEPA rooms), and microbial contamination (HEPA-LAF rooms only) (NF-90351).
With regards to expected neutropenia and duration, NIA high-risk patients (allogenic stem cell transplantation, acute myeloid leukaemia (AML) induction, acute lymphoblastic leukaemia) are hospitalized in HEPA-LAF rooms, and standard-risk patients (high-dose melphalan, non transplant AML) in HEPA rooms.
We performed an internal air and surfaces microbial sampling program in the high-risk unit to assess the global background level of Aspergillus, as air and surfaces were not sampled outside HEPA-LAF rooms.

Results
External controls demonstrate compliance in HEPA-LAF rooms for particle counts and microbial contamination, and in HEPA rooms for particle counts.
Internal sampling program in high-risk unit included 16 air settle plates and 55 surface contact plates. All samples were negative for fungal contamination in HEPA-LAF rooms (in and outside LAF areas). One air and one contact sample were positive respectively for *Aspergillus niger* (1 CFU/m^3) and *Aspergillus fumigatus* (1 CFU/plate) in the locker room. Two contact samples were positive in two anterooms (out of three), respectively for *Aspergillus fumigatus* (1 CFU/plate) and Aspergillus nidulans (1 CFU/plate)

Conclusion
On the whole, the quality control assessment (particle counts and microbial sampling) of the environment in our hematology unit appears satisfactory. An evaluation of these parameters is foreseen on a twice a year basis, as a baseline for extended assessment of fungal environmental contamination (cut-off determination) along with the prospective registration of NIA.

P.60 Haematopoietic stem cell transplantation in a patient with WHIM syndrome and pancytopenia: status at +1y

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Introduction
WHIM syndrome (OMIM#193670) is a rare primary immunodeficiency syndrome characterized by warts, hypogammaglobulinemia, infections and myelokathexis. It is caused by heterozygous mutations in CXCR4, leading to gain of function and increased CXCR4-CXCL12 signaling.

Objective
To describe a female adult patient with WHIM syndrome presenting with pancytopenia for which she received hematopoietic stem cell transplantation (HSCT).

Methods: A 19-year old patient was diagnosed with WHIM syndrome based on typical clinical constellation. A mutation c.956_957delCT (p.Ser319CystsX24) leading to frameshift and premature stop was identified in CXCR4. At the age of 19.5 y, she developed severe pancytopenia. Bone marrow examination showed myelofibrosis grade 2-3. No other cause (toxic, infectious, hemophagocytosis, genetic (JAK2) etc) was identified. Because the patient was transfusion dependent and because of extensive genital, oral and cutaneous warts, HSCT with a matched unrelated donor was undertaken. Conditioning regimen consisted of treosufan 42g/m^2 - fludarabine 150 mg/m^2 - ATG7.5mg/kg. PBMC derived CD34(+) cell dose was 10.75x10*6/kg. GvHD propylaxis consisted of ciclosporin and metothrexate.

Results
Patient is now 1y2m post -HSCT and alive and well. Neutrophils and thrombocytes engrafted at D+15.Because the HPV lesions flared ciclosporin was stopped at day + 72. There were no clinical signs of GvHD. Pancytopenia was corrected and myelofibrosis was not visualised on the bone biopsy at +1y post- HSCT. There is full donor chimerism.

Conclusion
A patient with WHIM syndrome and pancytopenia based on myelofibrosis is described. She received allo-HSCT. At 1y post-HSCT she is alive and well, pancytopenia is cured and HPV lesions are regressing slowly.

P.61 Graft-versus-host disease is a rare and tricky complication after autologous haematopoietic stem cell transplantation

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Graft-versus-host disease (GvHD) is a common complication after allogenic hematopoietic stem cell transplantation (Allo-SCT). Although extremely rare, this complication might occur after autologous hematopoietic stem cell transplantation (Auto-SCT). Two cases have been diagnosed in our institution during the last year.
The first observation was a 53 year-old female with a diagnosis of IgG Kappa multiple myeloma. She received high dose melphalan following by Auto-SCT in very good partial response in January 2012. She developed a pruriginous rash on day + 22 along with fever, hyperesinophilia and diarrhea. Skin biopsy was compatible with the diagnosis of GvHD. Gut biopsy was not specific. High dose steroids allowed a complete remission.
The second observation was a 66 year-old male with a diagnosed relapsed diffuse large B-cell lymphoma. He received abdominal radiotherapy and high dose BEAM chemotherapy following by Auto-SCT in partial remission in February 2010. He achieved a complete remission. In May 2012, he developed jaundice with abnormal liver function tests. Work-up include liver biopsy which showed GvHD features. Steroids administration led to a significant improvement.
GvHD after auto-SCT is a rare diagnosis and its physiopathology is mainly based on an auto-immune reaction. Literature is poor and therapy is based on GvHD after allo-SCT. Steroids remain the best first-line therapy. Nevertheless, further studies are warranted to better understand and manage this rare complication.

P.62 Isolated sphenoid fungal sinusitis as a rare cause of vision loss in a patient undergoing stem cell transplantation

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Invasive fungal sinusitis is becoming increasingly common in patients undergoing PBSCT and BMT. However isolated sphenoid fungal sinusitis is extremely rare and presentation is non-specific, making diagnosis delayed, management non-standardised and outcome poor.
We present a 67-year old female undergoing a second allogeneic stem cell transplantation with a matched unrelated donor for a relapsed acute myeloid leukaemia. Four days after PBSCT she complained of an unicranial headache non-responsive to analgetics. CT scan of the brain was negative. One week later she developed ptosis of the left eye with vision loss and failing eye tracking movements in all directions concluding to paralysis of the II, III, V and VI left cranial nerves. CNS fluid tested negative for infection. Urgent MRI of the brain showed isolated acute sinusitis of the left sphenoid sinus with extension to the sinus cavernosus. Serial galactomannan testing ad that time showed a remarkable increase from 0.18 to 2.35 over one week of time. Therapy with Voriconazole was initiated early in the course followed by a sphenoidectomy. Histological examination of the mucosal tissue demonstrated a massive amount of hyphae and on culture an extensive growth of Aspergillus fumigatus confirming the suspected diagnosis of invasive fungal sinusitis (IFS). The patient recovered well with disappearence of the headache and slow improvement of n III, V and VI. However there was no recovery of vision at the time of discharge.
In conclusion IFS is a rare disease largely attributable to aspergillosis in patients with BMT and hematological disease. Early physical findings are non-specific and ambiguous making diagnosis delayed and mortality high (50% to 80%). Introduction of serial Aspergillus galactomannan antigen test may provide early evidence of IFS. Systemic antifungal therapy combined with aggressive surgical debridement should be emphasised despite prolonged neutropenia and bleeding tendencies.

P.63 Successful treatment of late recurrence of mucormycosis with atypical presentation after haematopoietic stem cell transplantation

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Mucormycosis is a rare fungal infection, and is virtually only seen in immunocompromised patients. The mortality rate is high, especially in haematopoietic stem cell transplant (HSCT) recipients. We present here a case which demonstrates that efforts made can pay off.
A 55-year old farmer was treated at our institution for an acute myeloid leukaemia, diagnosed in September 2010. Medical history was insignificant. Conventional chemotherapy was followed by an allogeneic HSCT because of unfavourable cytogenetics. The early post transplant period was uncomplicated, and the leukaemia remained in complete remission. However, three months after HSCT he was admitted with a pulmonary infection, a Rhizopus mucormycosis. Combination therapy with ABLC and Posaconazole was started, and lobectomy was performed because of necrotising pneumonia. Soon after there was clinical improvement and six weeks later he could be discharged.
Two months after discharge the patient, in good general condition,

developed a renitent swelling on the right hemithorax, diagnosed as a seroma. CT-scan showed invasion of the thoracic wall and lytic lesions of several ribs. Surgical biopsy was scheduled, with per operatory conversion to partial resection of the thoracic wall because of extensive osteomyelitis of multiple ribs. Cultures showed hyphae, suggestive of persistent mucormycosis. ABLC-therapy was reinstituted, as was local care with application of AmphoB.
As there was a favourable evolution of the wound and cultures remained negative, the patient could be discharged six weeks after surgery. Daily administration of ABLC was continued at home for two more months. Follow-up imaging showed stable lesions. The residual fistula was excised surgically. At the most recent control visit the patient was in very good condition, without any evidence of residual active fungal infection, and with stable morphological and molecular remission of the leukaemia with good graft performance, now 18 months post HSCT.
We conclude that there is hope for patients with a diagnosis of mucormycosis, even after HSCT, but that aggressive and long lasting therapy is mandatory.

Abstract posters stem cell white blood cells immunity (P.64-P.76)

P.64 MicroRNA profile of circulating CD4-positive regulatory T cells in human adults and the impact of differentially expressed microRNAs on the expression of two genes essential to their function

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Regulatory T cells (Tregs) are characterised by a high expression of IL-2 receptor a chain (CD25) and of forkhead box P3 (FOXP3), the latter being essential for their development and function. Another major player in the regulatory function is the cytotoxic T-lymphocyte associated molecule-4 (CTLA-4) that inhibits cytotoxic responses. However, the regulation of CTLA-4 expression remains less well explored. We therefore studied the microRNA signature of circulating CD4+ Tregs isolated from adult healthy donors, and identified a signature composed of fifteen differentially expressed microRNAs. Among those, miR-24, miR-145 and miR-210 were down regulated in Tregs compared to controls and were found to have potential target sites in the 3'UTR of FOXP3 and CTLA-4; miR-24 and miR-210 negatively regulated FOXP3 expression by directly binding to their two target sites in its 3'UTR. On the other hand, miR-95, which is highly expressed in adult peripheral blood Tregs, positively regulated FOXP3 expression via an indirect mechanism yet to be identified. Finally, we showed that miR-145 negatively regulated CTLA-4 expression in human CD4+ adult peripheral blood Tregs by binding to its target site in CTLA-4 transcript 3'UTR. To our knowledge, this is the first identification of human adult peripheral blood CD4+ Tregs microRNA signature. Moreover, unveiling one mechanism regulating CTLA-4 expression is novel and may lead to a better understanding of the regulation of this crucial gene.

P.65 Human natural CD8-positive Treg microRNA signature: effect on regulatory T cell associated-genes

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MicroRNAs (miRs) are an abundant class of evolutionarily conserved small non-coding RNAs that regulate gene expression post-transcriptionally by affecting the degradation and translation of target mRNAs. Regulatory T-cells (Tregs) are T-cells that specialise in the suppression of immune responses to prevent pathological responses towards self-antigens, and to maintain homeostasis. Tregs have been described to be involved in solid tumours and haematologic malignancies. Our group was the first to describe a microRNA signature in human natural CD4-positive Tregs and in peripheral blood CD4+CD25+CD127lowTregs. Importantly, for both signatures, we could show how the described miRs specifically regulate genes associated to regulatory T-cell function. Recently, human CD8+ Tregs have gained interest in cancer pathogenesis field.
We investigated the microRNA expression profile of purified natural CD8+CD25+Tregs and its impact on Treg-associated functional molecules. Intracellular FOXP3 and cell surface CTLA-4 expressions were determined by flow cytometry on each sample. Quantitative FOXP3 mRNA expression was measured by real-time PCR. The suppressive function of CD8+CD25+ was assessed in Mixed Leucocyte Reaction. A microRNA profiling was performed using TaqMan Low-Density Array (TLDA) and for each miR that was found differentially expressed, a validation by individual quantitative PCR was performed. A microRNA signature was identified for CD8+CD25+FOXP3+CTLA4+ nTregs, composed by 9 microRNAs differentially expressed. In this signature 3 miRs were upregulated and 6 miRs were downregulated. We found potential target sites for these microRNAs in the 3'UTR of important Treg-associated genes, among which, FOXP3, CTLA4, CCR4, ICOS, CD28, CCL4 and IL2RA. We could also show that this CD8 nTreg microRNA signature is biologically relevant: miR-31 and -335 specifically control FOXP3 expression, while miR-9 and -155 control CTLA-4 expression as shown in site-directed mutagenesis and dual-Luciferase reporter assay experiments.
We are pursuing the study of the biological relevance of this miRNA signature by studying its impact on other important Treg-associated genes in order to better understand the regulation of Treg function modulation and to potentially reveal new targets for immuno-therapy in immune disorders and cancer.

P.66 TCR-dependency of *in vitro* T cell maturation in OP9-DL1 cocultures

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Notch-signaling has a crucial role in T cell development. This has led to the development of a bone marrow stromal cell line, transduced with the Notch ligand delta-like 1 (OP9-DL1), which supports differentiation of hematopoietic stem cells to mature T cells. However, the mechanism of final maturation and positive selection of T cells *in vitro* remains to be elucidated. The system lacks thymic epithelial cells that present peptide-MHC complexes to the maturing T cell *in vivo*. We have shown previously that induction of human HLA-A2 on murine OP9-DL1 cells does not augment *in vitro* maturation efficiency. This suggests that MHC complexes, and consequently TCR signaling, might not be involved in maturation of T cells on OP9-DL1. To confirm these data, we explored the role of TCR signaling in the final maturation of T cells on OP9-DL1 by performing conditional knockdown experiments of linker for activation of T cells (LAT), a linker protein in proximal TCR signaling, that is essential for beta-selection as well as positive selection in murine knockout models. To validate the LAT shRNA, transcription of shRNA was induced at a stage before the first TCR

checkpoint (beta-selection) and the effect was measured by the generation of DP cells. Ten days after induction of the LAT shRNA, only 14% of induced cells showed a DP phenotype, compared to 36% of uninduced control cells. We then evaluated the role of TCR signaling at the T cell selection checkpoint: LAT knockdown was induced when cells had reached the DP CD3+ stage. For TCRgd+ cells, we observed fewer mature T cells when LAT was downregulated versus control (17% *vs* 35%). Few TCRab+ mature cells were present in both control and LAT-downregulated populations, but a similar trend was observed. Our data suggest that acquisition of a mature phenotype in OP9-DL1 cocultures is TCR mediated, at least for the TCRgd+ population.

P.67 Comparison of immune reconstitution after hematopoietic stem cell transplantation with FLU-TBI vs. TLI-ATG conditioning

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The impact of the type of reduced intensity conditioning regimen used on immune recovery after allogeneic hematopoietic cell transplantation (allo-HCT) is poorly determined. We analyzed immune reconstitution in patients enrolled in a BHS-HCT sponsorised randomized study comparing two non-myeloablative conditioning regimens for allo-HCT for which cell samples were prospectively collected.

The conditioning regimen consisted of either 2 Gy TBI with 90 mg/m fludarabine (=TBI arm, n=21), or 8 Gy TLI plus thymoglobulin (ATG) 7.5 mg/kg (=TLI arm, n=19). Median ages at HCT were 59 yrs and 61 yrs in the TBI and TLI arms, respectively. Written informed consent has been obtained for each patient included.

Absolute T cell counts were lower in the TLI arm than in the TBI arm on day 28 after HSCT (P=0.04) but not thereafter. Further, B cells, as well as CD4+, CD4+CD45RA+ and CD4+CD45RO+ T cell reconstitution lagged behind in the TLI arm compared to the TBI arm the first year after HCT (B cells: p=0.0295 and others: p>0.0001). In contrast, reconstitution of CD8+ T cells, NK cells, Tregs and iNKT cells were similar in both groups. For the thymic function, while sjTREC levels were higher in the TBI arm than in the TLI arm on day 100 (P=0.002) and on day 365 (not significant) after HCT, the increase in sjTREC levels from day 100 to day 365 was similar in the 2 groups. The diversity of the TCR repertoire was similar in the 2 groups of patients on day 100 after HCT. Finally, we found that ATG persists in patients up to 17 days after allo-HCT in TLI patients (median of [ATG] at day 17=0.62 mg/l and for one patient at day 20=0.53).

These results suggest that ATG may be responsible for the delay of immune reconstitution of CD4+ T cells in the TLI arm and probably destroyed grafted sjTREC+ T cells. Finally, TLI conditioning has no impact on immune regulatory populations (Treg and iNKT) after the transplantation.

P.68 Heterosexual HIV-1 Transmission is Associated with Allogeneic KIR/HLA Ligand Combinations Governing Natural Killer Cell Alloreactivity

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Killer-immunoglobulin-like receptors (KIR) regulate natural killer (NK) cells in a human leukocyte antigen (HLA)-dependent manner. KIR/HLA gene combinations at the level of the individual influence susceptibility to HIV-1 acquisition and disease progression. Allogeneic KIR/HLA mismatches improve survival of leukaemia patients after hematopoietic stem cell transplantation. In this study, we analysed the effect of allogeneic KIR/HLA mismatches on HIV-1 transmission in a West African population of HIV-1 discordant and concordant couples. HIV-1 discordant couples were characterised by recipient partners with homozygous KIR2DL2, and by a mismatched recipient partner KIR2DL1/HLA-C2 index partner HLA-C1/C1 combination expected to allow licensed missing self' NK cell killing of index partners' cells. HIV-1 concordant couples on the other hand were characterised by KIR2DL3 homozygous recipient partners with HLA-C1/C2 bearing index partners, resulting in a matched KIR/HLA combination expected to inhibit NK cell killing. *In vitro* co-cultures of healthy donor-derived NK cells and HIV-1 patient-derived CD4+ T-cells confirmed the involvement of these allogeneic KIR/HLA combinations in NK cell-mediated CD4+ T-cell killing. Our data suggest that KIR/HLA incompatibility between sexual partners confers protection against HIV-1 transmission and that this may be due to recipient NK cell-mediated killing of the HIV-1 infected partner's cells.

P.69 Identification of biomarkers of hemostatic, endothelial and immune function in sepsis

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The pathophysiology of sepsis is still poorly understood. Recent evidence indicate that after an initial hyperinflammatory and procoagulant state, a protracted phase of consumptive coagulopathy, endothelial cell dysfunction and immune suppression is ultimately responsible for mortality. Most patients survive the initial phase with antibiotherapy, but may later need targeted treatment of hypocoagulability and immune stimulation. The identification of biomarkers of haemostasis, microvascular status and immune function is thus needed for patient stratification and tailored therapy.

In this study, eight patients with documented sepsis were tested at inclusion and after one, two and three days together with 21 normal individuals. Platelet function was assayed using the Multiplate instrument under ADP, arachidonic acid, ristocetin, collagen and thrombin stimulation. Clot formation was monitored by rotational thromboelastometry (ROTEM) using 1:1000 Innovin dilution (Srensen protocol). Immune competence was evaluated by numeration of regulatory T cells and monocyte subpopulations, i.e., CD14++CD16- (classical), CD14++CD16+ (intermediate) and CD14+CD16++ (non-classical) monocytes. Expression of HLA-DR, CD163 and CX3CR1 was quantified in each monocyte subset. Circulating endothelial cells (CEC) and endothelial progenitor cells (EPC) were identified using a stringent protocol proposed by Case and colleagues (Curr. Protoc. Cytom., 52:9.33.1, 2010) with slight modifications.

With all agonists, platelet activation was amplified in septic patients compared to controls (P<.05). ROTEM assays revealed a delayed initiation of clot formation, enhanced clot propagation and hypofibrinolysis (all P<.05). As previously described by Monneret *et al*., the proportion of Treg was increased in sepsis (P<.05). All monocyte subsets were increased in sepsis patients, mostly the intermediate fraction (P<.05). MFI of HLA-DR was downregulated while expression of CD163 was higher in all fractions (P<.05).

Expression of CX3CR1 was lower in classical and intermediate monocytes (P<.05) but higher in non-classical monocytes (NS). CEC were largely decreased in sepsis patients (P<.05) and EPC were slightly increased (NS).
A large array of haemostatic, vascular and immune abnormalities are identified in sepsis patients. Work is in progress to establish correlations with clinical scores and outcomes.

P.70 Immune Reconstitution After Alternative Hematopoietic Stem Cell Transplantation: Comparison of Unrelated Cord Blood (CB) and Mismatched Unrelated Donor (mmUD) Stem Cell Transplantation (SCT)

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There is no consensus about the best alternative stem cell source when no suitable HLA-matched (un)related donor is identified. Data about immune recovery and infections risk after alternative SCT are limited. Here, we compared immune reconstitution after CB- *vs* mmUD-SCT.

Methods
Sixty-six patients who underwent SCT from either CB (n=30) or 9/10 HLA-mmUD (n= 36) at Saint-Louis Hospital (Paris) from 01/2005 to 12/2010 were evaluated. Immune reconstitution was prospectively assessed by flow cytometry on fresh blood samples collected at one month before and then at 3, 6, 12, 18, 24 and 30 months after SCT. The following phenotypes were studied: NK cells (CD3-CD56+); B cells (CD19+) and their nave (CD27-) and memory (CD27+) subsets; CD4+ and CD8+T cells and their nave (CD45RA+CCR7+), central memory (CM:CD45RA-CCR7+), effector memory (EM:CD45RA-CCR7-) and late effector memory (LEM:CD45RA+CCR7-) subsets as well as regulatory T cells (Treg:CD4+CD25+CD127low) and NKT cells (CD3+CD56+).

Results
Reconstitution of T cells was delayed in CB cohort compared with mmUD during the first 12months post-SCT (P<0.05), particularly for CD8+T cells subset (P<0.01). In opposite, NK cells recovered more rapidly during the first 6months after CB-SCT (P<0.01). B cells counts were also higher in CB recipients till 24months post-SCT (*P*=0.005). This resulted in significant differences in the pattern of immune circulating cells after SCT in CB and mmUD recipients, particularly at 3 months post-transplant (Fig.1). Concerning CD4+ and CD8+ T cells, the distribution between nave and memory subsets was different in CB and mmUD cohorts as T cells from CB were characterized by smaller proportion of nave and higher proportion of EM cells during early post-transplant period (P<0.01). B cells reconstitution was characterized by predominance of nave subsets in both cohorts.

Conclusion
In comparison with mmUD, SCT from CB was characterized by faster NK and B cells reconstitution but delayed T cells recovery, mainly for CD8+ and nave compartments. We are currently assessing if these patterns of immune reconstitution translated in different susceptibility for severe infections.

P.71 Swachman-Diamond Syndrome: Frequent misdiagnosis as Jeune Syndrome and other peculiarities

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Background
Shwachman-Diamond Syndrome (SDS) is a rare inherited disorder. The typical diagnostic triad (neutropenia, skeletal dysplasia and exocrine pancreatic insufficiency) is not always present at diagnosis. Aims: to review mutations and initial presentation in a Belgian cohort of patients with genetically proven Shwachman-Diamond Syndrome (SDS).

Methods
A retrospective study in eleven patients with SBDS mutations.

Results
In ten patients an SBDS mutation was identified in both alleles, patient eleven was heterozygous. The mean age at diagnosis was 2.9 years. All patients had exocrine pancreatic insufficiency. Radiological evidence of skeletal dysplasia was present in 9/10 studied. Neutropenia was present in 8/11 patients. Failure to thrive was demonstrated for all but P8. 2/3 patients experiencing cholestatic hepatitis required admission to ICU. Both had blood CMV PCR(+). The 3rd patient suffers from chronic liver failure due to liver fibrosis. 10/11 experienced recurrent infections (septicemia, respiratory tract infections, skin infections). Two patients had an episode of symptomatic (convulsions) hypoglycemia without satisfying explanation despite extensive metabolic analysis.
Three patients received a diagnosis of Jeune syndrome (one patient died of respiratory insufficiency) and 1/11 of hypobetalipoproteinemia prior to diagnosis of SDS. A metabolic disorder was first suspected in patient 11 because of hypertrophic cardiomyopathy. Two couples of siblings in our cohort showed an entirely different course.

Conclusion
SDS triad was present at diagnosis in only 6/9. A high index of suspicion is crucial. The peculiar misdiagnoses as Jeune syndrome is striking as are the episodes of symptomatic hypoglycemia and the suspected increased susceptibility to severe CMV disease.

P.72 Successful Autologous Stem Cell Transplantation in a Girl with Neuropsychiatric Systemic Lupus Erythematosus

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Introduction
Neuropsychiatric Systemic Lupus Erythematosus (NPSLE) is a rare, chronic auto-immune disorder characterized by either diffuse (e.g. encephalopathy, coma, depression and psychosis) or complex presentation (e.g. encephalopathy with stroke or seizure). Disturbances of the cognitive function are a frequent problem. Autologous hematopoietic stem cell transplantation (ASCT) has shown to be an effective approach in those patients with SLE who are resistant to standard therapy.

Case report
A 15-year old girl was referred with progressive symptoms of fatigue, weight loss and anorexia. These symptoms started at the

age of ten years. The following years symptoms worsened and she developed headaches, depressive thoughts with catatonic as well as obsessive-compulsive and psychotic behaviour with multiple suicidal attempatients. Laboratory investigations revealed leuco- and thrombopenia, as well as a positive ANF, with anti-ENA RNP-a, RNP 70-k and RNP-c, suggestive for an auto-immune disorder. MRI of the brain was normal, but EEG was abnormal and the skin biopsy revealed IgG-deposition along the basal membrane. Initial treatment with corticoids and hydroxychloroquine resulted in improvement. However, after three months symptoms worsened and were resistant to subsequent treatment (Corticoids, low dose cyclophosphamide, plasmapheresis and anti- CD20 monoclonal antibodies). Finally, an ASCT according to the EBMT- ASTIL guidelines was performed. PBSC harvest was done after Cyclophosphamide (2X2g/m).The conditioning regimen consisted of cyclophosphamide 200 mg/kg and ATG 7.5 mg/kg. There were no severe problems with the conditioning regimen. The ASCT resulted in a spectacular improvement of the neuropsychiatric disabilities of this patient.

Discussion
Patients with NPSLE who are refractory to conventional treatment can achieve clinical remission after ASCT. This improvement is due to immunological changes that can not be achieved with other treatments. Patients should be considered for ASCT if a) they are resistant to first line treatment b) there is a high mortality risk due to de NPSLE, in this case suicidal attempatients c) ASCT should be performed before irreversible organ damage has occurred.

P.73 The immunomodulating peptide thymosin alpha 1 has no effects on multiple myeloma evolution and on immune reconstitution

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Background
Thymosin alpha 1 (Ta1) is a thymic peptide with known immuno-modulating properties, including increase of immune reconstitution after stem cell transplantation (SCT), enhancement of anti-tumour responses and direct effects on cancer cells (up-regulation of MHC-I expression, decrease of proliferation). We thus wanted to study the effects of Ta1 on murine models of multiple myeloma (MM) and immune reconstitution after SCT.

Methods and results
We first studied the *in vitro* effects of Ta1 on two murine MM cell lines, MOPC315.BM (Balb/c background) and 5TGM1 cells (C57Bl/KalwRij background). No significant effect of Ta1 has been observed on MHC-I/II up-regulation (flow cytometry), viability (MTT tests) or proliferation (3H-thymidin incorporation) of these cells. Since anti-tumour responses are complex and difficult to transpose *in vitro*, we studied the *in vivo* effects of Ta1 in the MM models. Mice were intravenously injected with 2.5x10^5 MM cells and treated daily by subcutaneous injections with 0.4 mg/kg Ta1 or PBS. MM development was evaluated by bone marrow and spleen infiltration at sacrifice, and paraprotein quantitation. No significant, recurrent effect of Ta1 was seen on MM development. Moreover, no significant effect of Ta1 could be observed on solid tumour growth (subcutaneous injection of MOPC315.BM cells). Finally, we studied the effects of Ta1 on immune reconstitution in a humanized murine model using immunodeficient NSG mice, transplanted with 5x10^5 human hematopoietic stem cells (AC133). Immune cell reconstitution was studied by flow cytometry on blood samples and at sacrifice (105 days post-transplantation). No significant effect of Ta1 could be seen on immune reconstitution in this model.

Conclusions
No biological effects of Ta1 could be observed on MM development or immune reconstitution in the proposed murine models, in contradiction with the immunomodulatory properties attributed to this peptide in the literature.

P.74 Rothmund-Thomson Syndrome: Immuno-Osseous challenges

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Background
Rothmund-Thomson (RTS) syndrome is a rare autosomal recessively inherited genodermatosis. It is characterized by poikiloderma, small stature, skeletal and dental abnormalities, cataract, and an increased risk of cancer. The syndrome is caused by mutations in RECQL4 at 8q24.

Aims
To describe the osseous and immunologic features of three patients with genetically confirmed RTS.

Methods
Immunological investigation, x-ray imaging and bone densitometry were performed at time of the first visit to the combined rheumatology-immunology clinic.

Results
All patients had characteristic poikiloderma as well as thumb anomalies. They were born dysmaturely and presented with failure to thrive. Age at genetic diagnosis was 5y, 4y and 3y for P1, P2, P3. Osteopenia and abnormal metaphyseal trabeculation of bones were striking on the initial skeletal survey in all patients. Z-scores on DXA scan were -0.1, -1.1 and -1.2 for P1, P2, P3 respectively at presentation. The presentation in P2 was dramatic with six fractures in upper and lower extremities and subluxation of both radii.
All patients were suffering from recurrent chest infections. P1 had granulomatous skin inflammation following primo VZV infection. All patients have low switched memory B cells for age, P1 has IgG2 deficiency. P1 and P3 have IgM deficiency. P1 and P3 have specific polysaccharide antibody deficiency. Results are pending for P2. All receive prophylactic antibiotics. P1 is treated with subcutaneous immunoglobulin substitution.

Conclusion
RTS is a genodermatosis with variable clinical presentation and course. Our observation of severe bone abnormalities and associated immunodeficiency merits attention for optimal management of these patients.

P.75 Invasive Aspergillus pneumonitis as a first presentation of X-linked Chronic Granulomatous Disease (X-CGD)

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Introduction
Chronic granulomatous disease is a primary immunodeficiency disorder characterized by recurrent life-threatening bacterial and fungal infections and granuloma formation. CGD is caused by

defects in the phagocyte NADPH oxidase, which results in inability of phagocytes to destroy certain microbes.

Material and methods
We describe a 3-year old boy who presented with invasive *Aspergillus pneumonitis* as a first presentation of X - linked CGD.

Results
A 3 year-old patient was referred because of persistent fever since more than one week, respiratory distress and hypoxemia despite intravenous antibiotic treatment with 3rd generation cephalosporins. He had been admitted five days earlier with a pneumonia of the right middle lobe. Chest CT at referral showed diffuse infiltrates of unclear etiology. An invasive fungal pneumonia was suspected and therapy with broad - spectrum antibiotics, Trimethoprim-Sulfamethoxazole (TMP-SMX), voriconazole and caspofungin was installed. Bronchoalveolar lavage showed a + galactomannan, suggestive of invasive aspergillosis. Lung biopsy showed non-necrotizing granulomata. The patient could be discharged after eighteen days with Voriconazole monotherapy and TMP-SMX (as bacterial prophylaxis). Diagnosis of X-CGD was confirmed with an abnormal dihydrorhodaminetest. Genetic analysis showed a missense mutation in CYBB, a gene encoding for p91Phox. This is one of the five proteins that make up the NADPH oxidase complex. Hemato-poietic stem cell transplantation (HCT) is planned in the near future.

Conclusion
Life threatening infections, even at adult age, can be the first presentation of X linked CGD, even in the absence of any prior minor or major infection. Rapid diagnosis and aggressive treatment of infections are crucial for survival. Antimicrobial prophylaxis with TMP-SMX and Itraconazole can reduce the rate of severe infections. Nevertheless, successful HSCT remains the only definitive cure for CGD.

P.76 A 10-year single center study of adult patients with histiocytic disorders in Antwerp

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Introduction
Adult histiocytosis is a rare and diverse group of proliferative disorders, characterised by accumulation of antigen-processing cells. Histiocytes may attack skin, bone, liver, lung, spleen, and hematopoietic system. According to the World Health Association (WHO), they can be divided into three categories: Langerhans cell histiocytosis (LCH), non-Langerhans cell histiocytosis (non-LHCH) and malignant histiocytosis. The Euro Histio Net divides in LCH, hemophagocytic lymphohistiocytosis (HLH) and rare histiocytic disorders (RHD). Treatment often involves radiation and chemotherapy. This study was performed in order to investigate the clinical characteristics of adult patients with histiocytosis treated in our hospital during the last decade.

Materials and methods
Eleven patients, with a median age of 38 years, who were referred and diagnosed with histiocytic disorders in the Antwerp University Hospital from 2001-2012, were investigated in this study.

Summary
According to the WHO, three patients had LCH, seven non-LCH, and one malignant histiocytosis. According to Euro Histio Net three patients had LCH, 2 HLH, and 6 RHD. Skin lesions and cytopenia were the most common manifestations, followed by bone lesions and lymphadenopathy. Different types of treatment protocols were used including surgical excision, radiotherapy, chemotherapy, and stem cell transplantation. Three patients did not respond well to the treatment and died due to complications of their disease. Patients and families in Belgium can gain support by contacting Langerhans Cel Histiocytose Belgium vzw, which is the Belgian branch of the Histiocytosis Association, and The Histio Net project (www.histio.net). No information is known if the patients described in this study contacted these associations.

Conclusion
Adult histiocytosis can be problematic for haematologists because of the unknown etiologies and pathogenesis, variable classifications and subtypes, diagnostic difficulties, poor therapeutic responses with high mortality, and complications after different therapeutic protocols.
There is a strong need by Belgian physicians and scientists committed to improving the lives of patients with histiocytic disorders to conduct clinical and laboratory research into the causes. Collaboration with the Histiocyte Society can help in this task.

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