BSTH
Belgian Society on Thrombosis and Haemostasis

Belgian Journal of Hematology

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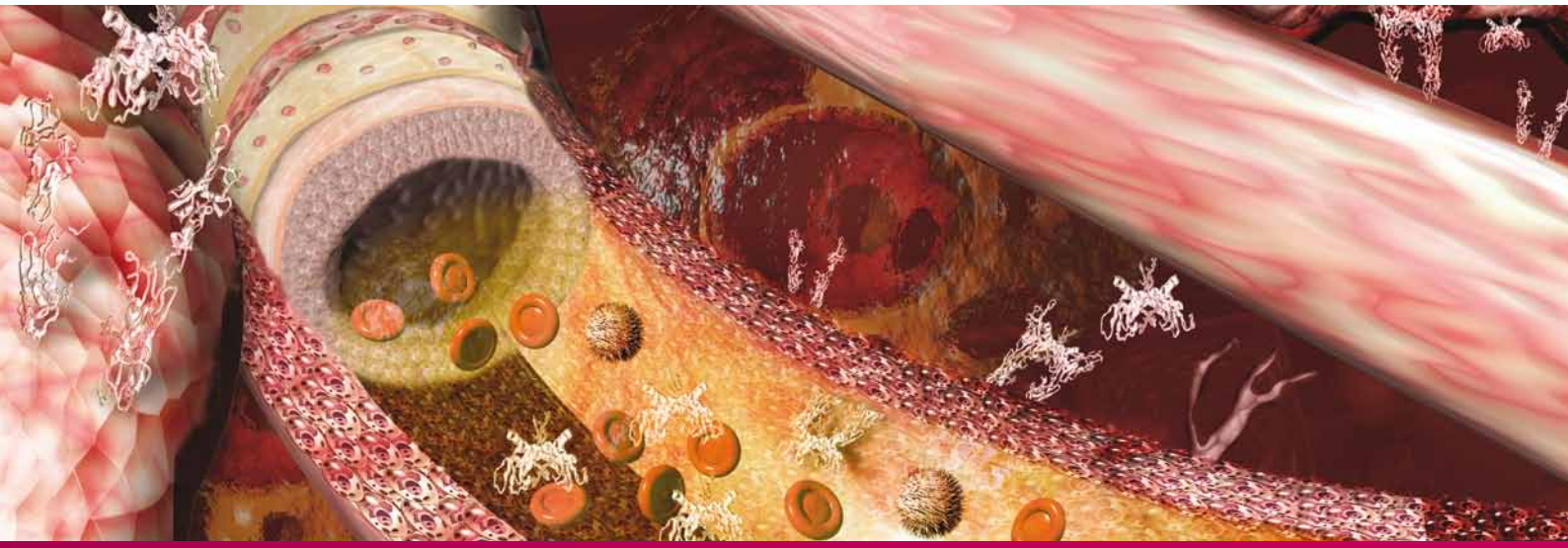
Belgian Hematological Society

The Belgian Journal of Hematology is the official journal of the Belgian Hematological Society (BHS), the Belgian Society on Thrombosis and Haemostasis (BSTH), the Belgian Society of Paediatric Haematology and Oncology (BSPHO) and the Belgian Society for Analytical Cytology (BVAC-ABCA)



Abstracts

29th General Annual Meeting of the Belgian Hematological Society

Gent, January 30 - February 1, 2014

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NEW **VELCADE SUBCUTANEOUS**

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• **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. • **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 hematopoëtische stamceltransplantatie 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 hematopoëtische stamceltransplantatie. VELCADE in combinatie met dexamethason, of met dexamethason en thalidomide, is geïndiceerd voor de inductiebehandeling van volwassen patiënten met niet eerder behandeld multipel myeloom, die in aanmerking komen voor een hooggedoseerde chemotherapie met een hematopoëtische stamceltransplantatie. **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. Tussen opeenvolgende doses van VELCADE moet minstens 72 uur zitten. Er zijn beperkte gegevens beschikbaar over herbehandeling met VELCADE. Dosering bij behandeling van progressief multipel myeloom: *Monotherapie:* VELCADE 3,5 mg poeder voor oplossing voor injectie wordt toegediend via een intraveneuze of subcutane injectie in de aanbevolen dosering van 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. Aanbevolen wordt om patiënten te behandelen met 2 VELCADE-cycli na het vaststellen van een complete remissie. Ook wordt aanbevolen om responderende patiënten die geen complete remissie bereiken in totaal met 8 VELCADE-cycli te behandelen. Tussen opeenvolgende doses van VELCADE moet minstens 72 uur zitten. *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.

Dosering bij patiënten met niet eerder behandeld multipel myeloom, die niet in aanmerking komen voor een hematopoëtische stamceltransplantatie: *Combinatietherapie met melfalan en prednison:* VELCADE 3,5 mg poeder voor oplossing voor injectie wordt toegediend via intraveneuze of subcutane injectie in combinatie met oraal melfalan en oraal prednison, zoals getoond in tabel 2. Een periode van 6 weken wordt beschouwd als een behandelcyclus. Er worden negen VELCADE behandelcycli toegediend. 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. Tussen opeenvolgende doses van VELCADE moet minstens 72 uur zitten.

Tabel 2: 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 met melfalan en prednison: Vóór het begin van een nieuwe behandelcyclus: - het aantal plaatjes moet ≥ 70 x 109/l en het absolute aantal neutrofielen moet ≥ 1,0 x 109/l zijn; - de niet-hematologische toxiciteit moet verminderd zijn tot graad 1 of baseline.

Tabel 3: Wijziging van de dosering in opeenvolgende cycli van VELCADE therapie in combinatie met melfalan en prednison

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.

Dosering bij patiënten met niet eerder behandeld multipel myeloom, die in aanmerking komen voor een hematopoëtische stamceltransplantatie: *Combinatietherapie met dexamethason:* VELCADE 3,5 mg poeder voor oplossing voor injectie wordt toegediend via een intraveneuze of subcutane injectie in de aanbevolen dosering van 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. Er worden vier behandelcycli met VELCADE toegediend. Tussen opeenvolgende doses van VELCADE moet minstens 72 uur zitten. Dexamethason wordt oraal toegediend in een dosis van 40 mg op de dagen 1, 2, 3, 4 en de dagen 8, 9, 10, 11 van de VELCADE-behandelcyclus. *Combinatietherapie met dexamethason en thalidomide:* VELCADE 3,5 mg poeder voor oplossing voor injectie wordt toegediend via een intraveneuze of subcutane injectie in de aanbevolen dosering van 1,3 mg/m^2 lichaamsoppervlakte, twee keer per week gedurende twee weken op de dagen 1, 4, 8 en 11, gevolgd door een 17 daagse rustperiode op de dagen 12 28. Deze periode van vier weken wordt beschouwd als één behandelcyclus. Er worden vier behandelcycli met VELCADE toegediend. Aanbevolen wordt dat patiënten met ten minste een partiële repons twee extra cycli ontvangen. Tussen opeenvolgende doses van VELCADE moet minstens 72 uur zitten. Dexamethason wordt oraal toegediend in een dosis van 40 mg op de dagen 1, 2, 3, 4 en de dagen 8, 9, 10, 11 van de VELCADE-behandelcycli. Thalidomide wordt oraal toegediend in een dosering van 50 mg per dag op de dagen 1-14 en als het wordt verdragen, wordt de dosis daarna verhoogd tot 100 mg per dag op de dagen 15-28. Vervolgens kan het verder worden verhoogd tot 200 mg per dag.

Tabel 4: Dosering van combinatietherapie met VELCADE bij patiënten met een niet eerder behandeld multipel myeloom die in aanmerking komen voor een hematopoëtische stamceltransplantatie.

Vc+ Dx	**Cycli 1 tot en met 4**				
	Week	**1**	**2**	**3**	
	Vc (1,3 mg/m^2)	Dag 1 -- -- Dag 4	Dag 8 -- -- Dag 11	rustperiode	
	Dx 40 mg	Dag 1 Dag 2 Dag 3 Dag 4	Dag 8 Dag 9 Dag 10 Dag 11	-	
Vc+Dx+T	**Cyclus 1**				
	Week	**1**	**2**	**3**	**4**
	Vc (1,3 mg/m^2)	Dag 1 -- -- Dag 4	Dag 8 -- -- Dag 11	Rustperiode	Rustperiode
	T 50 mg	Dagelijks	Dagelijks	-	-
	T 100 mga	-	-	Dagelijks	Dagelijks
	Dx 40 mg	Dag 1 Dag 2 Dag 3 Dag 4	Dag 8 Dag 9 Dag 10 Dag 11	-	-
	Cycli 2 tot en met 4b				
	Vc (1,3 mg/m^2)	Dag 1 -- -- Dag 4	Dag 8 -- -- Dag 11	Rustperiode	Rustperiode
	T 200 mga	Dagelijks	Dagelijks	Dagelijks	Dagelijks
	Dx 40 mg	Dag 1 Dag 2 Dag 3 Dag 4	Dag 8 Dag 9 Dag 10 Dag 11	-	-

Vc=VELCADE; Dx=dexamethason; T=thalidomide / a. De dosis thalidomide wordt alleen verhoogd tot 100 mg vanaf week 3 van cyclus 1 indien 50 mg wordt verdragen en tot 200 mg vanaf cyclus 2 indien 100 mg wordt verdragen. / b. Tot 6 cycli kunnen worden gegeven aan patiënten die ten minste een partiële respons bereiken na 4 cycli.

Dosisaanpassingen bij patiënten die in aanmerking komen voor een transplantatie: Voor dosisaanpassingen van VELCADE bij neuropathie, zie tabel 1. Als VELCADE wordt gegeven in combinatie met andere chemotherapeutica, dienen bovendien geschikte dosisverlagingen voor deze middelen overwogen te worden in het geval van toxiciteit, overeenkomstig de aanbevelingen in de Samenvatting van de Productkenmerken. 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/m^2 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 (zie tabel 5).

Tabel 5: 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. Er zijn geen studies naar het gebruik van VELCADE bij oudere patiënten met niet eerder behandeld multipel myeloom, die in aanmerking komen voor hooggedoseerde chemotherapie met een hematopoëtische stamceltransplantatie. Daarom kunnen voor deze populatie geen doseringsaanbevelingen worden gedaan. *Pediatrische patiënten:* De veiligheid en de werkzaamheid van VELCADE bij kinderen jonger dan 18 jaar zijn niet vastgesteld. 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. Tussen opeenvolgende doses van VELCADE moet minstens 72 uur zitten. *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 voor één van de vermelde hulpstoffen. Acute diffuse infiltratieve pulmonale en pericardiale aandoening. Als VELCADE in combinatie met andere geneesmiddelen wordt gegeven, raadpleeg dan de Samenvatting van de Productkenmerken van die geneesmiddelen voor additionele contra indicaties. **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, posterieur reversibel encefalopathiesyndroom, acute diffuse infiltratieve longaandoeningen en zelden autonome neuropathie. Samenvattende tabel met bijwerkingen: De bijwerkingen in tabel 6 hebben volgens de onderzoekers minstens een mogelijke of waarschijnlijke causale relatie met VELCADE. Deze bijwerkingen zijn gebaseerd op een integraal gegevensbestand van 5.313 patiënten van wie 3.515 patiënten werden behandeld met VELCADE in een dosis van 1,3 mg/m^2 en zijn opgenomen in tabel 6. In totaal werd VELCADE voor de behandeling van multipel myeloom toegediend aan 3.493 patiënten. De bijwerkingen worden hieronder per orgaansysteem en frequentie vermeld. De frequenties zijn 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 6 is gegenereerd met gebruik van versie 14.1 van de MedDRA. Postmarketing-bijwerkingen die niet werden gezien in klinische studies zijn ook toegevoegd. *Tabel 6: 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: bacteriële infecties*, virale infecties*, sepsis (incl. septische shock)*, bronchopneumonie, herpesvirusinfectie*, herpes-meningo-encefalitis#, bacteriëmie (incl. stafylokokken), hordeolum, griep, cellulitis, hulpmiddelgerelateerde infectie, huidinfectie*, oorinfectie*, stafylokokken infectie. Zelden: meningitis (incl. bacterieel), Epstein-Barr-virusinfectie, herpes genitalis, tandinfectie*, tonsillitis, mastoïditis, postvirale vermoeidheidssyndroom. Neoplasmata, benigne, maligne en niet-gespecificeerd (inclusief cysten en poliepen): Zelden: neoplasma maligne, plasmacytaire leukemie, niercelcarcinoom, gezwel, mycosis fungoides, neoplasma benigne*. **Bloed- en lymfestelsel-aandoeningen:** Zeer vaak: trombocytopenie*, neutropenie*, anemie*, leukopenie*. Vaak: lymfopenie*. Soms: pancytopenie*, febriele neutropenie, coagulopathie*, leukocytose*, lymfadenopathie, hemolytische anemie#. Zelden: uitgezaaide intravasculaire stolling, trombocytose*, hyperviscositeitssyndroom, bloedplaatjesaandoening NAO, trombocytopenische purpura, bloedziekte NAO, hemorragische diathese, lymfocytaire infiltratie. Immuunsysteem-aandoeningen: Soms: angio oedeem#, overgevoeligheid*. Zelden: anafylactische shock, amyloïdose, 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: dehydratie, hypokaliëmie*, hyponatriëmie*, bloedglucose abnormaal*, hypocalciëmie*, enzymafwijking*. Soms: tumorlysissyndroom, niet goed groeien*, hypomagnesiëmie*, hypofosfatemie*, hyperkaliëmie*, hypercalciëmie*, hypernatriëmie*, urinezuur abnormaal*, diabetes mellitus*, vochtretentie. Zelden: hypermagnesiëmie*, acidose, verstoorde elektrolytbalans*,vochtopstapeling, hypochloremie*, hyperchloremie*, hypovolemie, hyperfosfatemie*, metabole stoornis, vitamine-B-complex-deficiëntie, vitamine-B12-deficiëntie, jicht, verhoogde eetlust, alcoholintolerantie. **Psychische stoornissen:** Vaak: stemmingsaandoeningen en stoornissen*, angststoornis*, slaapaandoeningen en stoornissen*. Soms: psychische stoornis*, hallucinatie*, psychotische stoornis*, verwarring*, rusteloosheid. Zelden: zelfmoordgedachte*, aanpassingsstoornis, delirium, verminderd libido. **Zenuwstelsel-aandoeningen:** Zeer vaak: neuropathieën*, perifere sensorische neuropathie, dysesthesie*, neuralgie*. Vaak: motorische neuropathie*, bewustzijnsverlies (incl. syncope), duizeligheid*, dysgeusie*, lethargie, hoofdpijn*. Soms: tremor, perifere sensimotorische neuropathie, dyskinesie*, cerebellaire coördinatie- en evenwichtsstoornissen*, geheugenverlies (excl. dementie)*, encefalopathie*, posterieur reversibel encefalopathiesyndroom#, neurotoxiciteit, insultaandoeningen*, postherpetische neuralgie, spraakstoornis*, restless legs-syndroom, migraine, ischias, stoornis van aandacht, reflexen afwijkend*, parosmie. Zelden: cerebrale bloeding*, intracraniale bloeding (incl. subarachnoïdaal)*, hersenoedeem, transient ischaemic attack, coma, autonoom zenuwstelsel stoornis, autonome neuropathie, craniale verlamming*, paralyse*, parese*, presyncope, hersenstamsyndroom, cerebrovasculaire aandoening, zenuwwortellaesie, psychomotorische hyperactiviteit, ruggenmerg compressie, cognitieve aandoening NAO, motore disfunctie, zenuwstelselaandoening NAO, radiculitis, kwijlen, hypotonie. **Oogaandoeningen:** Vaak: oogzwelling*, gezichtsvermogen afwijkend *, conjunctivitis*. Soms: oogbloeding*, ooglidinfectie*, oogontsteking*, oculaire hyperemie, diplopie, droog oog*, 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: dysacusis (incl. tinnitus)*, gehoor beschadigd (t/m doofheid), oorongemak*. Zelden: oorbloeding, vestibulaire neuronitis, ooraandoening NAO. **Hartaandoeningen:** Vaak: tachycardie*. Soms: harttamponade#, cardiopulmonale stilstand*, hartfibrilleren (incl. atrium), hartfalen (incl. linker- en rechterventrikel)*, aritmie*, palpitaties, angina pectoris, pericarditis (incl. pericardeffusie)*, cardiomyopathie*, ventriculaire dysfunctie*, bradycardie. Zelden: atriale flutter, myocardinfarct*, atrioventriculair blok*, hart- en vaataandoening (incl. cardiogene shock), torsade de pointes, angina pectoris instabiel, aandoeningen van de hartklep*, kransslagaderinsufficiëntie, sinusstilstand. **Bloedvat-aandoeningen:** Vaak: hypotensie*, orthostatische hypotensie, hypertensie*. Soms: cerebrovasculair accident#, diepe veneuze trombose*, hemorragie*, tromboflebitis (incl. oppervlakkige), circulatoire collaps (incl. hypovolemische shock), flebitis, overmatig blozen*, hematoom (incl. perirenaal)*, slechte perifere circulatie*, vasculitis. Zelden: perifere embolie, lymfoedeem, bleekheid, erythromelalgia, vasodilatatie, verkleurde vene, veneuze insufficiëntie. **Ademhalingsstelsel-, borstkas- en mediastinum-aandoeningen:** Vaak: dyspneu*, bloedneus, bovenste/onderste luchtweginfectie*, hoesten*. Soms: longembolie, pleurale effusie, pulmonaal oedeem (incl. acuut), alveolaire bloeding#, bronchospasme, chronisch aspecifieke respiratoire aandoening*, hypoxemie*, luchtwegcongestie*, hypoxie, pleura-ontsteking*, hik, rhinorroe, dysfonie, piepen. Zelden: respiratoir falen, acuut ademhalingsnoodsyndroom, apneu, pneumothorax, atelectase, pulmonale hypertensie, haemoptysis, hyperventilatie, orthopnoea, pneumonitis, respiratoire alkalose, tachypnoea, longfibrose, bronchiale aandoening*, hypocapnie*, interstitiële longziekte, longinfiltraat, keelbeklemming, droge keel, toegenomen luchtweguitscheiding, keelirritatie. **Maagdarmstelsel-aandoeningen:** Zeer vaak: misselijkheids- en braaksymptomen*, diarree*, obstipatie. Vaak: gastro-intestinale bloeding (incl. mucosa)*, dyspepsie, stomatitis*, abdominale distensie, orofaryngeale pijn*, maagdarmstelselobstructie (incl. ileus)*, abdominale pijn (incl. gastro-intestinale en miltpijn)*, mondaandoening*, flatulentie. Soms: pancreatitis (incl. chronisch)*, hematemesis, lipzwelling*, abdominaal ongemak, orale ulceratie*, enteritis*, gastritis*, gingiva-bloeding, gastro-oesofageale refluxziekte*, colitis (incl. Clostridium difficile-colitis)*, ischemische colitis#, maagdarmontsteking*, dysfagie, prikkelbare darmsyndroom, gastro-intestinale aandoening NAO, tong beslagen, maagdarmstelselmotiliteitsaandoening*, speekselklieraandoening*. Zelden: pancreatitis acuut, peritonitis*, tongoedeem*, ascites, oesofagitis, cheilitis, fecale incontinentie, anussfincteratonie, fecaloom*, maagdarmstelselulceratie en perforatie*, gingivale hypertrofie, megacolon, rectale afscheiding, orofaryngeale blaarvorming*, 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, cytomegalovirushepatitis, leverbloeding, cholelithiase. **Huid- en onderhuid-aandoeningen:** Zeer vaak: rash*. Vaak: pruritus*, erytheem, dermatitis*, droge huid. Soms: erythema multiforme, urticaria, acute febriele neutrofiele dermatose, toxische huideruptie, toxische epidermale necrolyse#, stevens-johnsonsyndroom#, haaraandoening*, petechiae, ecchymose, huidlaesie, purpura, huidgezwel*, psoriasis, hyperhidrose, nachtzweet, decubitusulcus#, acne*, blaar*, pigmentatieaandoening*. Zelden: huidreactie, Jessner's lymfocytaire infiltratie, palmoplantair erytrodysesthesiesyndroom, subcutane bloeding, livedo reticularis, huidverharding, papel, fotosensitiviteitsreactie, seborrhoea, koud zweet, huidaandoening NAO, erytrose, huidulcus, nagelafwijking. **Skeletspierstelsel- en bindweefsel-aandoeningen:** Zeer vaak: skeletspierstelselpijn*. Vaak: spierspasmen*, pijn in extremiteiten, spierzwakte. Soms: spiertrekkingen, gewrichtszwelling, artritis*, gewrichtsstijfheid, myopathieën*, zwaar gevoel. Zelden: rabdomyolyse, temporomandibulair gewrichtssyndroom, fistel, vochtophoping in gewricht, pijn in kaak, botaandoening, skeletspierstelsel- en bindweefselinfecties en ontstekingen*, synoviumcyste. **Nier- en urineweg-aandoeningen:** Vaak: nierfunctie verminderd*. Soms: acuut nierfalen, chronisch nierfalen*, urineweginfectie*, urinewegtekenen en symptomen*, hematurie*, urineretentie, urinelozingsaandoening*, proteïnurie, azotemie, oligurie*, pollakisurie. Zelden: blaasprikkeling. **Voortplantings-stelsel- en borstaandoeningen:** Soms: vaginale bloeding, geslachtsorgaanpijn*, erectiele disfunctie. Zelden: testiculaire aandoening*, 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*, slijmvliesaandoening*, malaise*. Soms: algemene deterioratie van lichamelijke gezondheid*, gezichtsoedeem*, borstkaspijn, loopstoornis, het koud hebben, extravasatie*, kathetergerelateerde complicatie*, verandering in dorstgevoel*, borstkasongemak, gevoel van verandering in lichaamstemperatuur*, injectieplaatspijn*. Zelden: dood (incl. plotse), multi orgaanfalen, bloeding injectieplaats*, hernia (incl. hiatus)*, afgenomen genezing*, ontsteking, injectieplaatsflebitis*, gevoeligheid, ulcus, prikkelbaarheid, niet-cardiale pijn op de borst, pijn op de katheterplaats, gevoel van vreemd lichaam. **Onderzoeken:** Vaak: gewicht verlaagd. Soms: hyperbilirubinemie*, proteïneanalyses abnormaal*, gewicht verhoogd, bloedtest abnormaal*, C-reactief proteïne verhoogd. Zelden: bloedgassen abnormaal*, elektrocardiogram abnormaal (incl. QT verlenging)*, internationale genormaliseerde ratio abnormaal*, maag pH verlaagd, plaatjesaggregatie verhoogd, troponine I verhoogd, virusidentificatie en -serologie*, urineanalyse abnormaal*. **Letsels, intoxicaties en verrichtingscom-plicaties:** Soms: vallen, kneuzing. Zelden: transfusiereactie, breuken*, rigors*, aangezichtsletsel, gewrichtsletsel*, brandwonden, 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. *Perifere neuropathie bij combinatieschema's:* In studies waarin VELCADE werd toegediend als inductiebehandeling in combinatie met dexamethason (studie IFM 2005 01), en met dexamethason-thalidomide (studie MMY 3010), was de incidentie van perifere neuropathie in de combinatieschema's als in onderstaande tabel staat weergegeven:

Tabel 7: Incidentie van perifere neuropathie tijdens inductiebehandeling, opgesplitst naar toxiciteit en beëindiging van de behandeling als gevolg van perifere neuropathie

	IFM 2005 01		MMY 3010	
	VDDx (N=239)	VcDx (N=239)	TDx (N=126)	VcTDx (N=130)
Incidentie van PN (%) Alle graden van PN PN ≥ graad 2 PN ≥ graad 3	 3 1 < 1	 15 10 5	 12 2 0	 45 31 5
Beëindiging als gevolg van PN (%)	< 1	2	1	5

VDDx=vincristine, doxorubicine, dexamethason; VcDx=VELCADE, dexamethason; TDx=thalidomide, dexamethason; VcTDx=VELCADE, thalidomide, dexamethason; PN=perifere neuropathie. N.B.: Perifere neuropathie omvatte de voorkeurstermen 'neuropathie perifeer', 'perifere motorische neuropathie', 'perifere sensorische neuropathie' en 'polyneuropathie'.

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. *Herbehandeling van patiënten met gerecidiveerd multipel myeloom:* In een studie waarin herbehandeling met VELCADE werd toegepast bij 130 patiënten met gerecidiveerd multipel myeloom, die eerder ten minste een partiële respons vertoonden op een regime met VELCADE, waren de meest voorkomende bijwerkingen van alle graden, voorkomend bij minstens 25% van de patiënten trombocytopenie (55%), neuropathie (40%), anemie (37%), diarree (35%) en obstipatie (28%). Perifere neuropathie (alle graden) en perifere neuropathie ≥ graad 3 werden waargenomen bij respectievelijk 40% en 8,5% van de patiënten. Melding van vermoedelijke bijwerkingen: Het is belangrijk om na toelating van het geneesmiddel vermoedelijke bijwerkingen te melden. Op deze wijze kan de verhouding tussen voordelen en risico's van het geneesmiddel voortdurend worden gevolgd. Beroepsbeoefenaren in de gezondheidszorg wordt verzocht alle vermoedelijke bijwerkingen te melden via: **België:** Federaal agentschap voor geneesmiddelen en gezondheidsproducten, Afdeling Vigilantie, EUROSTATION II, Victor Hortaplein, 40/ 40, B-1060 Brussel, Website: www.fagg.be. e-mail: adversedrugreactions@fagg-afmps.be. **Nederland:** Nederlands Bijwerkingen Centrum Lareb, Website: www.lareb.nl. **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:** 31/07/2013. *Meer informatie is beschikbaar op verzoek.*

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INTRODUCTION

Dear members, colleagues, friends,

The Belgian Hematological Society welcomes you to the 29th General Annual Meeting (GAM) in Ghent!

Hematologists, scientists, nurses, data and quality managers, corporate partners and patients will have the great opportunity to exchange their knowledge and experience in the field of hematology.

We have tried to be as broad as possible in covering non-oncological topics as well as major topics in hemato-oncology and laboratory hematology. Based on the good experience of last year, the committees meeting on Thursday 30th will combine updates on the committee's activities with short educational "hot topics" presented by Belgian experts in the field.

In addition, on Thursday 30th, the patient organization "CMP" related to multiple myeloma and Waldenström's macroglobulinemia will organize its 10th anniversary symposium with educational and interactive sessions and the participation of the French-speaking sister organization "MyMu".

We are particularly honored to welcome the BVAC/ABCA and BSPHO societies, which will join us and organize their annual meetings simultaneously on Friday 31st. It is with great pleasure that we again welcome the simultaneous "Nurse symposium" on Friday 31st which has become a tradition, unique to hematology with increasing interest and enthusiasm.

During the main program on Friday 31st and Saturday 1st, participants will deal with a great variety of topics presented, during highlight sessions, in keynote lectures and company-sponsored satellite symposia. In line with our yearly tradition, excellent international leaders in the field will give top quality lectures. The future of hematology will be touched upon during a very promising "New Horizons" session on Saturday morning. Young researchers will present their work during the Poster session with a commented poster walk and the best abstracts will be presented in a plenary session on Friday afternoon. Awards will be granted on Saturday morning.

Another new tradition since a few years is the "Dinner and party" on Friday evening. This year a very exciting "funky music group" will entertain young and less young hematologists… Don't miss the event!

Finally, during our short "General Annual Business Meeting" on Saturday morning, we will report on the Society's activities and election's results.

The board is looking forward to meeting you all in Ghent!

Rik Schots
President of the BHS

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Program BHS committees meeting — Thursday 30 january 2014

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Ann Janssens

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Chantal Doyen

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Lucien Noens

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Friday 31 january 2014

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Friday 31 january 2014

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10.15 Discussion

10.30 - 11.00 Coffee break

10.30 - 19.00 Poster view

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Sponsored by Celgene
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9.30 New treatment options in lower-risk MDS
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Chairmen: J. Maertens and V. Robin

To BEACOPP
M. André, Namur

Not to BEACOPP
M. Federico, Modena, Italy

10.30 - 11.00 Coffee break

11.00 - 12.45 **New horizons in hemato-oncology**
Chairmen: T. Kerre and D. Selleslag

11.00 Next generation gene sequencing
A. Kohlmann, München, Germany

11.30 Haploidentical stem cell transplantation in 2014
A. Velardi, Perugia, Italy

12.00 CAR T cells
S. Riddell, Seattle, USA

12.45 - 13.00 Closing, awards and results board elections
R. Schots

Abstracts invited lectures

Management of Sickle Cell Disease

A. Ferster, Hôpital Universitaire des Enfants Reine Fabiola (ULB), Brussels

Sickle-cell disease (SCD) is one of the most common severe genetic diseases worldwide with more than 300 000 affected children born every year. With an incidence of less than 1:2000 live births, SCD is the most common genetic disease to be diagnosed in Brussels. Polymerization of hemoglobin S is the *primum movens* responsible for all the clinical features observed in this disease i.e. vaso-occlusion, chronic anemia and hemolysis leading to the development of a vasculopathy. Reduced NO bioavailability, increased neutrophils and reticulocytes adhesion to activated endothelial cells, proinflammatory environment and hypercoagulable state are also involved in the pathophysiology of SCD. SCD is a multisystemic disease marked not only by acute crisis but also chronic damage with progressive organ failure, chronic pain in a background of increased susceptibility to infections. Pain is the hallmark of SCD. The frequency and severity of vaso-occlusive crises (VOC) vary widely both among patients and over time for each patient. Effective treatment of acute pain is one of the most challenging problems in the management of SCD. Pain, infections, worsening of anemia, acute chest syndrome, cerebral vasculopathy and pulmonary hypertension may complicate the course of SCD.
Neonatal screening with early implementation of anti-pneumococcal prophylaxis was an essential step that improved SCD children survival to 95%. Neonatal screening for hemoglobinopathies has been adopted at a national level in US, England, France, and since 2007 in the Netherlands but is not yet generalized in Belgium.

The management of SCD includes many different aspects: infectious prophylaxis, education and counselling, management of acute events (VOC, acute chest syndrome, acute anemia), prevention of stroke in children and management of chronic problems. Comprehensive care also includes education, genetic counseling, and patient's empowerment for successful transition to adulthood.
Current indications for acute and chronic transfusion are prevention of stroke and perioperative complications, acute chest syndrome, and acute anemia. Repeated/ chronic transfusions encompass the risk of iron overload, alloimmunization, and delayed hemolytic transfusion reactions. The prevalence of RBC alloimmunization in patients with SCD is high but slightly reduced with extended RBC antigen matching. In specific stituations, molecular blood group typing should improve RBC matching and prevent the development of alloimmunisation.
Hydroxyurea (the only FDA-approved drug for SCD) treatment is associated with a reduction in the rates of pain, acute chest syndrome, hospitalizations and blood transfusions, improves hematologic values and reduces hemolysis. Interestingly it might preserve organ function and recent studies report an improvement in overall survival.

The only curative approach to SCD is hematopoietic stem cell transplantation. The results of transplantations performed with grafts from an HLA-identical sibling donor and after myeloablative conditioning in young patients are excellent. New reduced-toxicity conditioning regimens for older patients and transplants with alternative donors, including related haploidentical donors are in development in experimental settings.

In conclusion, the management of patients with SCD is complex and requires a multidisciplinary approach. As life expectancy continues to improve, transition to adult care becomes a critical issue.

Old and new anticoagulants in practice

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The development and validation of new oral anticoagulants (NOACs) targeting either thrombin (dabigatran etexilate) or factor Xa (rivaroxaban and apixaban) for the prevention and treatment of thrombosis represent one of the most important therapeutic advances in the field of thrombosis and haemostasis of the last decades. NOACs have major pharmacologic advantages over vitamin K antagonists (VKA), including rapid onset/offset of action, fewer drug interactions, and predictable pharmacokinetics, eliminating the requirement for regular coagulation monitoring. Regulatory agencies have approved several NOACs for specific indications based on the results of clinical trials demonstrating efficacy and safety that are at least as good, if not better, than VKA (for stroke prevention in atrial fibrillation and treatment and secondary prevention of venous thromboembolism) or low-molecular-weight heparin (LMWH), which is injectable (for initial treatment of venous thromboembolism and thromboprophylaxis in patients undergoing hip or knee arthroplasty). Several factors could limit the systematic adoption of this new therapeutic class into clinical practice including concerns regarding medication adherence without laboratory monitoring, uncertainty about dosing in some patient populations (eg, renal dysfunction, marked extremes of body weight), and higher drug costs compared with warfarin. Other issues are the current absence of specific antidotes for NOACs and assays to measure drug levels at most centers. Although the indications for NOACs will likely expand in the future, they have so far not been validated in several conditions requiring therapeutic or preventive anticoagulation. As practitioners gain familiarity with the drugs and healthcare systems adapt to their use, NOAC use will likely increase substantially over time. VKA and LMWH, however, will continue to remain appropriate and validated anticoagulants of choice for many patients.

Practical approach of patients with mild bleeding disorders

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As a preamble let's say that in this summary we will focus on congenital bleeding disorders and not the acquired ones (in the oral presentation some aspects of acquired mild bleeding disorders will also be discussed). Mild bleeding disorders (MBD) which are more frequent than severe bleeding disorders are linked with many problems as mentioned below.
The first problem when we speak about MBD is the definition. Indeed none is accepted by the specialists in haematology. Usually it is a definition by exclusion, i.e. that a patient presenting unusual mild bleeding symptoms and not having a severe platelet disorders (Glanzmann thrombasthenia, Bernard Soulier syndrome) or coagulation disorders (severe haemophilias A and B, von Willebrand type 3, afibrinogenemia, FX and FXIII severe deficiencies) is considered as a mild bleeder. But it is important to add immediately that some patients with a genetic proven defect leading to a severe deficiency may phenotypically behave as mild bleeders and the reverse is true.
The second problem lies in the perception of the patient and/or the physician of what they feel as mild bleeding. Indeed when a "normal" population is asked if they have bleeding symptoms, up to half of them will report to have some kind of bleeding problems. Therefore a systematic approach is proposed now with various bleeding assessment tools and bleeding scores in order to avoid performing useless laboratory exams. Scores to better evaluate menorrhagias are also of help. However all validated scores (most

of them having been originally designed for von Willebrand diseases) have some limitations.
The third problem concerns the laboratory work-up. As mentioned a thorough clinical approach (familial, personal bleeding history, sites of bleeding, spontaneous of provoked nature of the bleeding, haemostatic challenges, drugs intake, etc.) with scores should first be done. Next comes the choice of tests. We propose a two step approach in case of a suspicion of MBD. The first time we see the patient and we think a MBD is possible we perform a complete blood count (anemia?), blood group genotyping, PT, aPTT, TT, von Willebrand and fibrinogen levels and according the results of these screening tests we perform detailed coagulation assays. We see the patient a second time and we repeat dubious results plus we complete this work-up when necessary by platelet assays (essentially aggregation and secretion studies) as well as FXIII and antiplasmin assays. There is a discussion right now at the ISTH subcommittee whether some platelet assays should not be performed as a first step. Global assays such as thrombin generation time or thromboelastograms as well as the search for collagen disorders are also discussed.
The fourth problem is related to treatments. Indeed the reason for doing such a work-up is to give specific treatments. But here we are faced with at least two difficulties, first not to administer unnecessary treatments to patients (risks of allergy, alloimmunization, inhibitors, thrombosis, etc.) and second, in almost half of the patients, no laboratory anomaly is discovered (patients classified as mild bleeders having bleeding of undefined cause). In these patients empirical treatments with tranexamic acid and DDAVP should be carefully discussed.
In our lecture we will detail a MBD retrospective study we did in Geneva as well an ongoing prospective study.

Breaking Pathologic Signaling in Myeloproliferative Neoplasms: Beyond JAK2 Inhibitors

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BCR-ABL negative Human Myeloproliferative Neoplasms (MPNs) are associated with a highly prevalent activating mutation in the pseudokinase domain of JAK2, JAK2 V617F, which is present in >70 of patients, as well as with activating mutations in the gene coding for the thrombopoietin receptor (TpoR, c-MPK) (present in 5-8% of patients), and at low frequency with mutations inactivating negative regulators of the JAK-STAT pathway. Thus, MPNs are diseases of the JAK-STAT pathway, especially of JAK2. Inhibitors of JAK2, although not discriminating between JAK2 V617F and wild type JAK2 demonstrated efficacy in reducing constitutional symptoms and splenomegaly in myelofibrosis, and one molecule is now used in the clinic for the treatment of myelofibrosis. We have recently shown that JAK2 and PI3-kinase inhibitors synergize to inhibit MPN progenitor proliferation, both in vitro and in preclinical models, indicating that cytokine-independent proliferation in MPNs reflects an addiction to the PI3-kinase pathway.
In this presentation, first I will discuss evidence from our laboratory and several others pointing to potential mechanisms by which mutants in the calreticulin gene appear to be activating the JAK-STAT pathway and induce MPN, with a predilection for the megakaryocytic lineage. Such mutants of calreticulin loose the KDEL endoplasmic reticulum retention signal and are associated with the majority (>60% of MPNs negative for JAK2/MPL mutants). Second, I will present data showing that constitutive active STAT5, which is a hallmark of pathologic signaling via JAK2 V617F and TpoR W515 mutants induces genes different from those induced by cytokine-activated STAT5, and recruits p53 to novel sites, not physiologically targeted by p53. The role of p53 in chronic and accelerated phase of MPNs will be discussed, especially in the light of high p53 mutant prevalence in secondary acute myeloid leukemia. Finally, signaling from pathologically activated JAK-STAT pathway will be integrated with effects of inactivating mutations in epigenetic regulators, like TET2 and EZH2 and their potential roles in evolution of MPNs to secondary acute myeloid leukemia.

To BEACOPP or not to BEACOPP: That is the question

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How to treat patients with advanced Hodgkin Lymphoma (HL)? Starting with ABVD or with escalated bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone (BEACOPPesc)? There is a general agreement that BEACOPPesc is associated with better disease control in terms of response rate, progression free survival and freedom from treatment failure. However, there is no agreement on the superiority of BEACOPPesc over ABVD in terms of Overall Survival (OS). According to the results of the German Hodgkin Study Group HD15 trial, a 5 year OS of roughly 95% is achievable in advanced stage HL patients treated with six cycles of BEACOPPesc. Moreover, a recently published network meta-analysis performed by the German Hodgkin Study Group including 10,111 patients with advanced-stage HL showed an OS benefit of 10% at 5 years for BEACOPPesc over ABVD. The authors concluded that an OS difference of that magnitude was obviously clinically relevant and provides substantial progress for patients. However, in the trial conducted by the Fondazione Italiana Linfomi comparing BEACOPP with ABVD and high dose therapy as salvage therapy in both arms, no advantage in the 7 year OS rate was observed (89% vs. 84%, p=0.39). Again, in the EORTC 20012 Intergroup trial, the 4 year OS was not improved with BEACOPP (90.3% vs. 86.7%, p=0.208). Finally, BEACOPPesc is generally considered too much toxic for patients older than 60 years. Thus, the superiority of BEACOPPesc over ABVD is still matter of debate.
On the other hand, data emerging from several randomized controlled trials of a response-oriented approach driven by early PET assessment and the potential of brentuximab vedotin combinations to improve the efficacy of ABVD with an acceptable toxicity profile, allow us to conclude that the recommendation to give all patients six cycles of BEACOPPesc upfront is not appropriate. Although more effective in the short and medium period, the role of BEACOPPesc in the management of patients with advanced stage HL has to be better defined yet, and is most likely that can be spared to the majority of patients.
Investigators should continue looking for the best position for BEACOPPesc in the overall treatment strategy, switch to this regimen only in patients not responding to two initial courses of ABVD, or addressing to BEACOPPesc only patients recognized at very high risk of initial failure according to the current and future available prognostic indexes.
In conclusion, patients with advanced HL need to be fully informed of the various opportunities to cure, their likelihood of success, and short- and long-term toxicity costs of each approach. The decision about which path to choose should be shared with the well-informed patient.

Implementing next-generation deep-sequencing assays in diagnostic algorithms in hematological malignancies

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Next-generation sequencing (NGS) platforms have evolved to enable an accurate and comprehensive detection of molecular mutations in heterogeneous tumor specimens. Initial research

efforts applying massively parallel sequencing methods had focused on examining so-called index patients to investigate the landscape of molecular mutations in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). These studies led to the discovery of key mutations in genes such as *IDH1, DNMT3A, BCOR, RAD21*, and unraveled the involvement of the dysregulated splicing machinery in various types of hematological malignancies *(SF3B1, SRSF1, U2AF1, ZRSR2)*. Consequently, molecular biomarkers will soon no longer be sequenced individually. Instead, panels of markers will be assessed in a massively parallel way with high sensitivity and multiplexing many patients per analysis. A great challenge will be the implementation of such a novel methodology into existing laboratory workflows. Here, we present potential applications of this assay and in particular will focus on the utility of amplicon deep-sequencing in characterizing myeloid and lymphoid neoplasms where the number of molecular markers applied for disease classification, patient stratification into differing risk groups, and individualized monitoring of minimal residual disease is constantly increasing. We will discuss many facets of this assay that need to be taken into account, e.g. the preparation of sequencing libraries with molecular barcodes, specific experimental design options when considering sequencing coverage to calculate diagnostic sensitivity, or the use of suitable software and data processing solutions to obtain accurate results. Taken together, amplicon deep-sequencing has already demonstrated a promising technical performance that warrants the further development towards a routine application of this next-generation sequencing technology in diagnostic laboratories so that an impact on clinical practice can be achieved. Besides being able to address the ever growing demands for high-throughput clinical research topics, NGS will be particularly useful to provide molecular information for many disease areas in the cost-effective manner and fast turn-around time that individualized treatment regimens will be requiring.

Abstracts oral presentations O.1 - O.8

O.1 ABVD (8 cycles) vs. BEACOPP (4 escalated cycles => 4 baseline) in stage III - IV low risk Hodgkin Lymphoma (IPS 0-2): final results of LYSA H34 trial

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Escalated BEACOPP achieved superior time to treatment to failure over ABVD in patients with disseminated Hodgkin lymphoma. However, later clinical trials have failed to confirm Overall Survival (OS) superiority over ABVD.

Methods

We compared ABVD (8 cycles) vs. BEACOPP (escalated 4 cycles => baseline 4) in patients with International prognostic score (IPS) ranging 0-2. Primary endpoint was Event Free Survival. Patients with IPS >2 were included in the EORTC Intergroup 20012 study.

Results

One hundred fifty patients were randomized (ABVD 80, BEACOPP 70): median age 28 y, males 50%. IPS was 0-1 for 64%. CR was 85% for ABVD and 90% for BEACOPP. Progression or relapses were more frequent in ABVD (17 vs 5 patients). With a median follow-up of 5.5 years, 7 patients died : 6 in ABVD and 1 in BEACOPP (HL 3 & 0, 2nd cancer 2 & 1, accident 1&0). EFS at 5 yrs was estimated at 62 % vs. 77 %, respectively (HR = 0.6, p=0.07). Progression Free Survival at 5 yrs was 75 % vs. 93 % (HR = 0.3, p=0.007). Overall suvival at 5 yrs was 92 vs. 99 % (HR = 0.18, p=0.06).

Conclusion

EFS and OS were not different between treatment arms. However, more progressions/relapses were observed with ABVD. As in high risk group, additional considerations as late morbidity due to salvage treatment may help decisions making toward ABVD or BEACOPP for low risk patients.

O.2 Establishment of a murine graft-versus-myeloma model using allogeneic stem cell transplantation

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Multiple myeloma (MM) is a malignant plasma cell disorder with poor long-term survival and high recurrence rates. Despite evidence of graft-versus-myeloma (GvM) effects, the use of allogeneic stem cell transplantation (allo-SCT) has remained controversial in MM. In the current study, we investigated the anti-myeloma effects of allo-SCT from B10.D2 mice into MHC-matched myeloma-bearing Balb/cJ mice (previously injected with the MOPC315.BM myeloma cell line), based on a chronic graft-versus-host disease (GvHD) murine model.

Balb/cJ mice were injected intravenously with luciferase-transfected MOPC315.BM cells, and received 30 days later an allogeneic (B10.D2 donor) or autologous (Balb/cJ donor) transplantation by intravenous administration of bone marrow cells and splenocytes. We observed a graft-versus-myeloma effect in 17 out of 18 allogeneic transplanted mice, as luciferase signal completely disappeared after transplantation, whereas 13 of the 13 autologous transplanted mice showed myeloma evolution. Lower serum paraprotein levels and myeloma infiltration in bone marrow and spleen in the allogeneic setting confirmed the observed GvM effect, while allogeneic mice also displayed chronic GvHD symptoms. Moreover, prior sensitization of B10.D2 donor mice with myeloma cells resulted in exacerbated GvHD symptoms in recipient mice, suggesting a cross-reactivity of responses directed against antigens present on myeloma cells and allo-antigens. *In vivo* and *in vitro* data suggest possible involvement of effector memory CD8 T cells in the GvM effect, reactive against both myeloma and normal Balb/cJ cells. Finally, the role of CD8 T cells was confirmed when CD8 T-cell depletion of the graft resulted in reduced GvM effects. In the CD8 T cell-depleted group, 4 out of 6 mice (66.7 %) showed strong bioluminescence signal and myeloma symptoms after transplantation, whereas in the standard allogeneic transplantation group, only one mouse out of 18 developed bioluminescence signal after transplantation (5.6 %).

We successfully established an immunocompetent murine graft-versus-myeloma model, involving effector memory CD8 T cells which display both anti-myeloma activity and alloreactivity. This model could be a basis for further studies assessing ways of dissociating GvM from GvHD.

O.3 Comparison of 2 nonmyeloablative regimens for allogeneic HCT: a phase II randomized study from the HCT committee of the BHS

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Background
The BHS transplantation committee initiated a phase II randomized study comparing nonmyeloablative HCT with either TBI-Flu or TLI-ATG conditioning. The primary endpoint was the 180-day incidence of grade II-IV acute GVHD. Here, we report the final analysis of the study.

Patients and methods
107 patients with hematological malignancies and a 10/10 allelic matched donor were randomized in the TBI (n=55) or TLI (n=52) arms. Ten patients (5 in each arm) were excluded from the analyses because they did not meet the inclusion criteria at the time of the start of the conditioning (disease relapse before the start of the conditioning (n=5), ineligible for further irradiation (n=3), donor inegibility (n=1), poor PS precluding transplantation (n=1)). Thus, the analyses included data from 97 patients randomized to the TBI (n=50) or TLI (n=47) arms. The 2 groups were well balanced in terms of age and disease risk. Postgrafting immunosuppression combined tacrolimus and MMF. Median follow-up for surviving patients was 45 (range, 19-65) months.

Results
Donor T-cell chimerism levels on days 180 and 365 and marrow chimerism levels on days 40 and 180 after HCT were lower in the TLI arm. Before day 180, 6/50 (12%) TBI patients had grade II (n=4), grade III (n=1) or grade IV (n=1) acute GVHD while 4/47 (9%) TLI patients had grade II (n=3) or IV (n=1) acute GVHD. Two-year Kaplan-Meier probability of moderate/severe chronic GVHD was 50% in the TBI arm versus 21% in the TLI arm (P=0.007). Finally, 3-year overall (OS) and progression-free (PFS) survivals were 58% and 56%, respectively, in the TBI arm, versus 61% and 36% (P=0.09), respectively in the TLI arm.

Conclusions
In comparison to patients conditioned with TBI, those receiving TLI experienced a similar incidence of acute GVHD but a lower incidence of chronic GVHD, as well as a trend for worse PFS but similar OS.

O.4 Functional characterization of activating mutants of the JAK3 tyrosine kinase implicated in tumoral transformation of T lymphocytes

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The JAK3 tyrosine kinase is a member of the family of Janus Kinases (or JAKs) that are intracellular kinases associated to cytokine receptors. These receptors lack intrinsic kinase activity and are dependent on JAKs for signal transduction. JAK3 is specifically associated with the common gamma chain shared by the receptor complexes for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Inactivating mutations in JAK3 lead to impaired T cell development causing Severe Combined Immunodeficiency Syndrome (SCID), whereas activating mutations, which induce a constitutive activation of cytokine signal transduction, have been described in various leukemia types.
While it has been previously shown that activating JAK1 and JAK2 mutants are dependent on the binding to a functional receptor complex for their activity, little is known about the functional characteristics of JAK3 mutants. Here, we show that the majority of JAK3 mutants need to associate to a functional cytokine receptor complex such as the IL-9 receptor composed of the common gamma chain, the IL-9Ra chain and JAK1, in order to activate STAT transcription factors. Interestingly, one of the mutants, JAK3-L857P, is not dependent on such receptor complexes for its activity. The introduction of a mutation in the FERM domain that abolishes the interaction with the common gamma chain does not affect the activity of JAK3-L857P, whereas the integrity of this domain is required for all other mutants. This differential requirement for functional cytokine receptor complexes paired with a distinct sensitivity to JAK inhibitors. Ruxolitinib, which blocks preferentially JAK1 and JAK2, abolished the proliferation of cells transformed by a JAK3 mutant that requires a functional receptor complex, but is much less potent on cells expressing JAK3-L857P. By contrast, the latter were more sensitive to JAK3-specific inhibitors. Taken together, our results show that different JAK3 mutations can induce constitutive STAT5 activation through distinct mechanisms and that a better understanding of these mechanisms could offer new therapeutic perspectives.

O.5 *In vivo* gene expression profiling in the murine 5T33MM model identifies epigenetic-regulated genes predictive for prognosis and drug-sensitivity

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Multiple myeloma (MM) is a plasma cell malignancy that until now remains incurable due to the development of resistance and refractory disease. Epigenetics plays a major role in MM pathogenesis and epigenetic modifying agents such as DNA methyltransferase (DNMTi) and histone deacetylase inhibitors (HDACi) are currently widely investigated. Using gene expression profiling (GEP) after *in vitro* treatment of 5 human cell lines with the DNMTi decitabine (DAC) and the HDACi trichostatin-A (TSA), we recently identified deregulated genes prognostic for MM patient' survival in the HM and UAMS-TT2 cohort. These genes were used to develop a score allowing the prediction of *in vitro* sensitivity of HMCLs and primary MM cells towards different DNMTi and HDACi. However, as the epigenetic response of MM cells may be influenced by the bone marrow niche, we now tried to validate these results *in vivo*. Therefore, 5T33MMvv inoculated mice were treated at established disease with sub-optimal doses DAC, the HDACi JNJ-26481585 (JNJ-585) or the combination for 5 days and GEP was performed. Using Significance Analysis of Microarray, expression of 166 (DAC), 493 (JNJ-585) and 310 (combo) genes were found to be significantly deregulated (Fold change $\geq$ 2 and FDR = 0) after treatment. Next, we identified 26 (DAC) and 134 (JNJ-585) genes with a prognostic value ($p<0.005$) in both the HM and UAMS-TT2 cohort. These prognostic genes were used to build a DNA methylation (DM) and histone acetylation (HA) risk score to predict survival of MM patients. We also identified respectively 2 and 7 genes that were significantly differentially expressed between DAC or HDACi sensitive versus insensitive primary MM cells (Fold change $\geq$ 2 and FDR = 0). In addition, we identified 19 (HDACi) and 38 (combo) genes in common with the genes obtained after treatment of the HMCL. From these lists, we selected 9 good prognostic genes to further investigate for their role in MM pathogenesis, potential use as biomarker for drug efficacy and prediction of sensitivity towards anti-myeloma agents.

O.6 Long-term survival in patients receiving rhEPO following allogeneic hematopoietic cell transplantation

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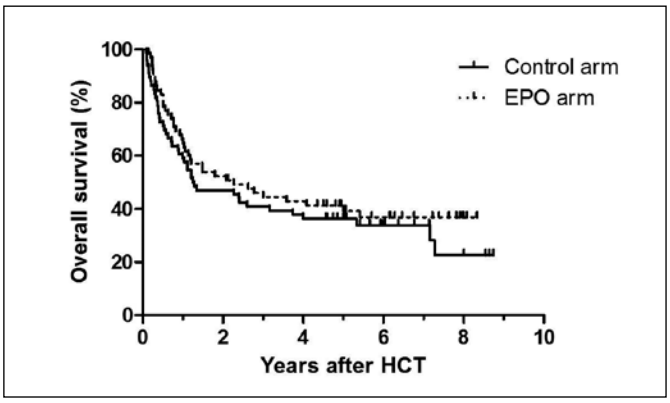
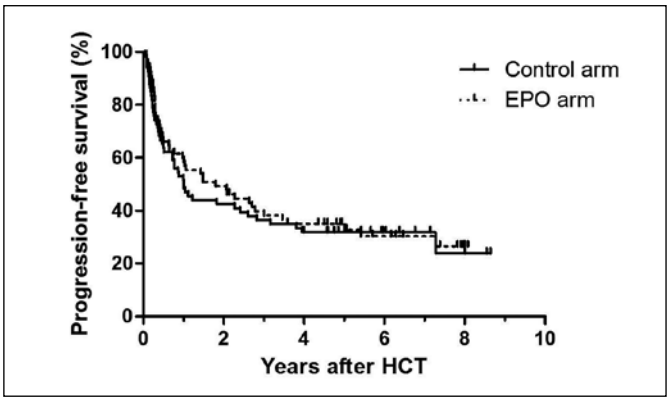
We recently reported the efficacy of erythropoietin therapy following allogeneic hematopoietic cell transplantation (allo-HCT) on erythroid reconstitution and transfusion requirements. As some meta-analyzes suggest increased mortality in patients with cancer receiving erythropoiesis-stimulating agents (ESA) particularly when no concomitant chemotherapy is administered, we assessed long-term follow-up of patients included in the study.
In this trial, 131 patients given myeloablative or non-myeloablative allo-HCT were randomized 1:1 between two arms: the control arm (no erythropoietic therapy) vs the EPO arm (erythropoietin β 500 U/kg/week). Patients were also stratified in 3 cohorts : 42 patients underwent myeloablative HCT (cohort A), while patients in the cohorts B (n=39) and C (n=50) were given non-myeloablative conditioning. RhEPO was administered once a week, from day 28 in cohorts A and B, whereas patients in cohort C received rhEPO from day 0. Treatment duration was initially planned for 16 weeks but there were rules for decreasing or withholding rhEPO according to the Hb level.
The total number of injections of rhEPO until day 126 was 12.6 ± 4.6. After day 126, 19 patients received maintenance therapy with the lowest possible dose of rhEPO. Median times to reach Hb ≥ 13 g/dL, ≥ 12 g/dL or +2 g/dL were shorter in the EPO arm and this resulted in a reduction of transfusion requirements in the EPO arm. After a median follow-up of 655 days, we did not observe any difference in rates of overall survival (OS). Indeed, 1-year and 5-year OS were 59% and 36% in the control arm and 65% and 39% in the EPO arm (p=0.33) (figure 1). Progression-free survival (PFS) was also similar in the two arms: 1-year and 5-year PFS were 48% and 31% in the control arm and 58% and 32% in the EPO arm (p=0.64) (figure 2).



O.6 *Figure 1.* Overall survival after HCT



O.6 *Figure 2.* Progression-free survival after HCT

In conclusion, rhEPO following allo-HCT did not have an impact on survival in long-term analyses.

O.7 NOTCH1 c.7544-7545 delCt mutation identifies a subgroup of lymphocytic leukemia patients with poor outcome

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NOTCH1 has been found recurrently mutated in a subset of patients with chronic lymphocytic leukemia (CLL). Recent studies showed that activating mutations of NOTCH1 proto-oncogene occur in about 10% CLL at diagnosis and are associated with an unfavorable clinical outcome (Rossi et al, 2012).
We have investigated 105 samples (collected between 2008-2013) of CLL patients for the NOTCH1 mutation: c.7544-7545 delCt. The NOTCH1 mutation was investigated by amplification refractory mutation system (ARMS) PCR of the CLL patients at diagnosis, with a median age of 65 years (range 40-87), 60 males and 45 females. Additionally other prognostic markers in CLL have been investigated. FISH analysis was performed for the detection of trisomy 12, deletion 11q, deletion 13q and deletion 17p. The IGHV gene mutational status was performed by DNA sequencing.
We found NOTCH1 c.7544-7545 delCt mutations in 16.2 % of the cases. The results of the NOTCH1 mutation analysis were compared with the results of the other prognostic factors as trisomy 12, deletion 11q, deletion 13q, deletion 17p and IGHV gene mutation status. All patients harboring a NOTCH1 mutation and consequently had a poor prognosis did not show a deletion 13q as the sole aberration which is connected with a good prognosis. Trisomy 12, deletion 11q, deletion 17p have been found in NOTCH1 mutated as well as in NOTCH1 wild type cases. Half of all cases show an unmutated IGHV gene and/or a c.7544-7545 delCt mutation which predicts a poor outcome.
NOTCH1 seems to be an independent predictive marker for poor outcome in CLL patients. Because of the importance as prognostic marker in CLL the NOTCH1 c.7544-7545 delCt analyses is included in our spectrum of tests for CLL patients. In the future the follow-up of the patients will give us more information of the clinical impact of the NOTCH1 mutation.

O.8 JAK2 V617F-Negative AND MPL W515K/L-Negative Essential Thrombocythemia: a High Resolution SNP Array Study

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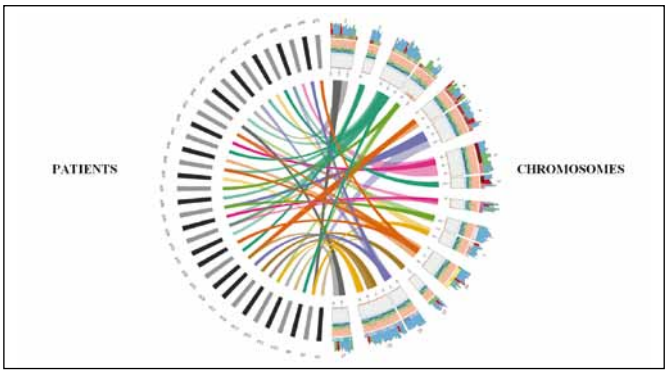
Background
JAK2 V617 and *MPL* W515K/L are the most common mutations in essential thrombocythemia (ET), occurring in approximately 60 % of cases. The molecular cause of the remaining ET cases is still largely unknown.

Aims
We sought to investigate *JAK2* V617F-negative and *MPL* W515K/L-negative ET for regions of copy number variations (CNV) and loss of heterozygosity (LOH).

Methods
We studied blood or bone marrow samples from a series of 64 *JAK2* V617F-negative and *MPL* W515K/L-negative ET cases. They



0.8 *Figure 1.* Circos plot showing the recurrent CN-LOHs of 35 ET patients

were subjected to 2.7M SNP array by Affymetrix and analyzed for recurrent CNVs or recurrent LOHs (≥3 Mbs).

Results
Only 8 recurrent gains were identified, in 5/64 patients. Interestingly, the most common gain, occurring in 5 cases was a gain of chr7 q22.3, including the gene encoding Nicotinamide phosphoribosyltransferase *(NAMPT).* NAMPT is known to be overexpressed in several cancers such as multiple myeloma. It catalyzes the rate-limiting step of the nicotinamide adenine dinucleotide (NAD+) biosynthesis pathway. By quantitative PCR (qPCR) on genomic DNA, we were able to validate the gain in 2/5 patients. Other recurrent gains involved regions of chromosomes 2, 5, 7, 12, 13, and 22. These gains included, amongst others, *LCP1* and *CYTIP.* The array data were also analyzed for recurrent LOHs on ChAS, yielding 17 recurrent copy neutral LOHs (CN-LOH) in 35 patients (circos plot). The most common CN-LOH region was on chromosome 3 appearing in 8 patients. Other CN-LOH regions involved chromosomes 1-7, 10, 12, 15, and 17.

Conclusions
In this series of 64 *JAK2* V617F-negative and *MPL* W515K/L-negative ET patients we found recurrent gains not reported previously in the database of genomic variants in only 8% of patients, and small areas of CN-LOH in ~55% of cases. However, most of the latter probably are constitutional. Our SNP array study provides further evidence that gains, losses or CN-LOH of small genomic regions do not play an essential role in the pathogenesis of the majority of *JAK2* V617F-negative and *MPL* W515K/L-negative ET.

Posters non malignant hematology P1.01-P1.12

P1.01 Flow cytometric screening in patients at risk for paroxysmal nocturnal hemoglobinuria and evaluation of clone sizes: a retrospective study

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Introduction
Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematopoietic stem cell disease which can lead to life-threatening complications. Patients with PNH may present with a wide range of signs and symptoms.

Aims and methods
The goal of our study was firstly to investigate in a larger cohort of patients the clinical presentations that alert physicians to demand flow cytometric PNH testing and secondly to further characterize PNH clone positive patients. Therefore, all PNH tests performed in our laboratory between January 2010 and December 2012 were retrospectively reviewed.

Results
A total of 271 PNH analysis (corresponding to 230 patients) were performed. Intramural samples encoded for 75% of all analysis. The reasons for testing were hemolytic anemia in 66 samples (24%), aplastic anemia (AA) or hypoplastic bone marrow (BM) in 43 (16%), myelodysplastic syndrome (MDS) in 32 (12%), unexplained cytopenia in 30 (11%), and unexplained thrombosis in 40 (15%). In the remaining 22% no clinical data were available. Thirty-nine out of 271 samples (14%, corresponding to 18 patients) showed a PNH clone: 12 had an AA/hypoplastic BM, 2 were diagnosed in patients with Coombs negative hemolytic anemia, 3 had a MDS and 1 was found in a patients with unexplained cytopenia. Clone sizes on white blood cells (granulocytes/monocytes) varied significantly (overall range from 0.05% to 98.6%): in classic PNH with massive hemolysis large clones sizes could be found (range: 2.7%-97%), in patients with subclinical PNH the clones sizes varied between 0.13% and 9%, and patients who had a PNH clone in the setting of BM failure syndromes showed highly variable clone sizes, ranging from 0.05% to 98.6%. Multiple PNH analyses (≥2 follow-up tests) were performed in 12 PNH clone positive patients: both stable and fluctuating clone sizes were seen over time.

Conclusion
Our data show that treating physicians ask flow cytometric PNH screening in all populations at risk and that follow-up testings are performed in PNH clone positive patients for evaluation of changes in clone size

P1.02 Acquired hemophilia: a retrospective evaluation in the Ghent University Hospital

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Introduction
Acquired hemophilia A (AHA) is a rare bleeding disorder caused by autoantibodies against factor VIII. We analyzed the characteristics and outcomes of the patients diagnosed with AHA in the Ghent University Hospital from January 2004 to November 2013.

Description
Sixteen patients (seventeen episodes) were diagnosed with AHA, twelve were treated and followed in the Ghent University Hospital and four in a peripheral hospital. The median age of onset was 72 years comparable with data in literature. The male: female ratio was 9:8.
We dispose of clinical data on twelve cases, they all presented with a bleeding event: skin, muscles, soft tissues or mucous membranes. At diagnosis the activated partial thromboplastin time was prolonged in all episodes with available measurements. The median FVIII at diagnosis was 1% (0-49%), with most of the patients (76%) presenting with FVIII<5%. The median inhibitor titer was 40 BU/mL, with a wide range (0.7-247 BU/mL, Bethesda assay). In 85% of patients no underlying disorder could be identified. One case was associated with pregnancy.

The basic therapeutic strategy involves control of the bleeding episodes with hemostatic agents and eradication of the autoantibodies by administration of immunosuppression. Recombinant activated FVII (NovoSeven®) was initiated in 54% of patients. Patients who reached complete remission (CR) -58%- all received corticosteroids. The inhibitor disappeared after a median duration

of 52 days. One patient relapsed (7% *vs* 33.5% in literature) after 14 months of CR. We found a higher mortality rate (42%) compared to the literature (7.9%-33%), probably due to the transfer of critically ill patients to the Ghent University Hospital.

Conclusion
The characteristics of the patients diagnosed with AHA in the Ghent University Hospital over a period of almost nine years are comparable with those described in the literature, except the lower relapse rate and higher mortality rate. AHA is a severe bleeding disorder with a potential fatal outcome.Not recognizing the signs and symptoms may delay diagnosis and adequate treatment with severe impact on prognosis.

P1.03 Comparison of hemoglobin on RapidPoint 500 blood gas analyser (Siemens) to XE-5000 cell counter (Sysmex)

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Objective
To evaluate whether hemoglobin concentration (Hb) measured by a RapidPoint 500 blood gas analyser (RP500) is interchangeable with that measured by a XE-5000 automated blood cell counter. Both methods use different blood tubes and different measurement principles.

Materials and Methods
Routinely prelevated heparinized arterial blood gas syringes (ABG) (Sarstedt, Germany) and EDTA vacutainer tubes (BD, USA) from patients admitted to the intensive care unit were collected. These tubes were prelevated simultaneously through the same arterial catheter. Hb on ABG was tested randomly on maximum five different RP500s (Siemens, Germany) at three hospital sites with a minimum of 30 measurements on each analyzer. Hb on EDTA tubes was measured by one XE-5000 (Sysmex, Japan). All samples were tested within 24 hours after blood sampling. Statistical analyses were performed using MedCalc (MedCalc Software, Belgium).

Results
During 18 days, 75 ABG and EDTA tubes were collected from 33 different patients. Hb on RP500 ranged from 6.4 to 14.3 g/dL and on XE-5000 from 5.9 to 13.3 g/dL. The overall correlation between Hb on XE-5000(x) and RP500(y) was y=0.4320+1.0425x with the correlation coefficient r= 0.9792 (95%CI[0.9714 to 0.9849]). Using the method of Bland and Altman, the overall mean difference in Hb between the XE-5000 and the RP500 was -0.84 g/dL (95%CI [-0.8946 g/dL to -0.7908 g/dL]) and among the RP500 analysers ranged between 0.02 g/dL and 0.16 g/dL .

Conclusion
Both methods correlate well but the RP500 overestimates Hb on 5 different analysers. Much smaller biasses were found among the RP500s. Correction factors at the level of the RP500 software should be established to make both measurements interchangeable. When reporting a parameter measured by different assay principles to clinicians, one should establish a correlation study. External QCs for Hb on blood gas analysers and interchangeable internal QCs between blood gas analysers and cell counters, could help in continuous quality assessment. Unfortunately, these QCs are not available.

P1.04 Multiple myeloma cells instruct myeloid-derived suppressor cells to release pro-angiogenic cytokines

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Multiple myeloma (MM) is a hematological malignancy, characterized by the accumulation of monoclonal plasma cells in the bone marrow. Myeloid-derived suppressor cells (MDSC) are immature myeloid cells that are implicated in cancer progression through immune suppressive effects and tumor supporting capacities. Two major sub-populations have been described: monocytic or « MO-MDSC », and granulocytic or « PMN-MDSC ». In this work, we examined the immune suppressive function and angiogenic profile of the two different MDSC subpopulations in an immunocompetent murine model of MM, using 5TGM1 myeloma cell line.
We isolated the two MDSC subpopulations from bone marrow (healthy or myeloma-diseased mice), using immunomagnetic bead assay (MACS). Both of the isolated MDSC fractions caused a dose-dependent suppression of lymphocyte proliferation, but PMN-MDSC showed significantly higher suppression than MO-MDSC. No significant difference was seen in the suppressive phenotype of MDSC from MM mice compared to healthy mice.
We studied the effects of MM MDSC on angiogenesis using a gelatin-sponge chorioallantoic membrane assay. We observed a significant increase of angiogenesis in the presence of PMN-MDSC from MM mice when compared with PMN-MDSC from healthy mice or negative control. When MDSC were implanted in combination with 5TGM1 cells on CAMs, we observed again significantly higher angiogenesis in the presence of PMN-MDSC (compared to 5TGM1 cells alone or negative control). In order to identify pro-angiogenic factors implicated in the observed effects, we evaluated the RNA expression level in MDSC from MM mice compared to healthy mice. Increases in Placental Growth factor (PlGF) and angiopoietin 2 (Ang2) were identified in MM MDSC compared to controls. After co-culturing MDSC and 5TGM1 myeloma cells in a transwell insert allowing passage of secreted cytokines, we confirmed an upregulation of PlGF and Ang2 (after 24h and 48h) in PMN-MDSC, as well as other pro-angiogenic genes. These results show that immunosuppressive PMN-MDSC and MO-MDSC are present in the 5TGM1 model of MM and reveal pro-angiogenic properties of PMN-MDSC in MM, probably implicating PlGF and Ang2.

P1.05 A multicentre study characterising different regimens for introducing second- or third-line anagrelide in 177 patients in France

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Introduction
Anagrelide is indicated in the European Union for at-risk patients with essential thrombocythaemia (ET) in whom prior therapy (PT) is not sufficiently effective or well tolerated.

Aim
To identify the switch modalities used when introducing anagrelide and determine any possible influence on 6-month outcomes (efficacy, tolerability and maintenance).

Methods
This observational study (NCT01192347) was conducted in 43 clinical centres across France. High-risk patients (aged >60 years; history of thrombohaemorrhagic events; platelet count >1000x 10^9/L) with ET were identified and enrolled within 1 month of switching to anagrelide, and monitored for a total of 6 months.

Results
177 patients were enrolled: 62% female, 76% aged >60 years, median baseline platelet count 553x10^9/L. Intolerance to therapy (65%) and inefficacy (41%) were the most frequent reasons for treatment switch (factors not mutually exclusive). Almost all patients switched to anagrelide from hydroxycarbamide (93%). The Summary of Product Characteristics (SPC)-recommended anagrelide starting dose (1mg/day) was used most frequently (53%); a notable proportion of patients (41%) started on 0.5mg/day, and starting doses ranged from 0.3 to 1.5mg/day. The median anagrelide dose at study end was 1.5mg/day.
The method of anagrelide introduction was consistent with the SPC dosing recommendations in 76% of patients. After 6 months' follow up, 85% of patients (n=144/170) were still receiving anagrelide and 71% (n=120/170) achieved a platelet response. 87% of patients who discontinued PT after initiating anagrelide achieved a platelet response (n=34/39) compared with 67% of patients who discontinued PT before anagrelide initiation (n=77/115). Platelet response rates were higher in patients whose anagrelide initiation was consistent (n=100/133, 75%) versus inconsistent (n=20/37, 54%) with the SPC dosing recommendations. The most frequent adverse drug reactions were palpitations (13%) and headache (11%).

Conclusions
This real-world evidence study showed that highest platelet response rates were observed when PT was discontinued after anagrelide initiation or when anagrelide was initiated consistently with the SPC dosing recommendations. Safety data corresponded with the SPC.

P1.06 An unusual presentation of tuberculosis

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Immune thrombocytopenia is characterized by accelerated platelet destruction and decreased platelet production. Platelet antibodies are detected in 60% of patients. Most cases are considered primary (no underlying cause), whereas a minority is drug-induced or attributed to an underlying condition including infection, lymphoproliferative disease or autoimmune disorders. The most common associated chronic infections are hepatitis C (HCV), Human immunodeficiency virus (HIV) and Helicobacter Pylori.
A 43-year-old female with sickle cell disease presented with mucocutaneous bleeding. Her medical history revealed pulmonary arterial hypertension, osteonecrosis and hepatic cholestasis as a result of vaso-occlusive crises.
Physical examination showed petechiae, ecchymoses and localized erythema nodosum on both legs. Laboratory work-up documented moderate anemia, marked thrombocytopenia and presence of platelet autoantibodies. Screening for viral infections (HIV, HCV), systemic lupus erythematosis and antiphospholipid syndrome was negative. Bone marrow examination was unremarkable.
PET-CT scan revealed isolated FDG-avid periportal adenopathies. Histopathological examination of a CT-guided lymphe node biopsy was consistent with tuberculous adenitis. Microbiologic culture identified Mycobacterium Tuberculosis.
She was successfully treated with antituberculous drugs and intravenous immunoglobulines. During follow-up, a sustained platelet count recovery was achieved. Absence of platelet autoantibodies was documented after completion of therapy.
We report immune thrombocytopenia as a rare manifestation of tuberculous adenitis. This case illustrates that testing, in a population at risk for an otherwise unsuspected persistent infection with tuberculosis, might be worthwhile since treatment of the immune thrombocytopenia (steroids, immunosuppressive agents) may worsen the infection.

P1.07 Jacobsen syndrome as a rare cause of neonatal thrombocytopenia

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Thrombocytopenia is one of the most common hematological problems in neonates, affecting 0,5-0,9% of all newborns and more than 25% of all patients admitted to neonatal intensive care units. In rare cases, it is caused by inherited conditions wherein decreased platelet count may be associa ed to a broad spectrum of clinical and biological symptoms including abnormal platelet functions. We report the case of a baby boy born at 36 weeks of gestation with intra-uterine growth retardation, facial dysmorphism (low-set and posteriorly rotated ears with hypoplastic helices, micrognathia, hypertelorism), perimembranous intraventricular septal defect and severe neonatal thrombocytopenia. The platelet count at birth was 10,000/uL and remained at 21,000/uL despite a treatment with intravenous immunoglobulin. The child received 2 transfusions of platelets and his count progressively increased around 50,000/uL at day 14. A bone marrow examination was performed at day 14 and revealed a megakaryocytopenia. Based on the dysmorphic syndrome, a genetic workup was rapidly initiated. The CGH-array revealed a deletion in the 11q24.2-q25 region consistent with the diagnosis of Jacobsen syndrome. The genetic investigation of the family was negative, suggesting that this deletion occurred *de novo*. The platelet count progressively increased and was normalized after 4 months of life. In addition, the child developed a moderate microcytic anemia currently treated by iron supplementation. His neutrophil and lymphocyte counts remained normal for the age over time. A first coagulation screening was normal but additional assessments of the platelet morphology and functions are scheduled within the next months. To date, over 200 cases of Jacobsen syndrome have been reported. This syndrome is caused by terminal deletion of the long arm of chromosome 11. It includes dysmorphic craniofacial features, cardiac and urogenital malformations, syndactyly, ophthalmologic problems, GH and thyroid defect, developmental delay and neonatal thrombocytopenia or pancytopenia. Platelet count tends to increase spontaneously to near normal level. The prevalence has been estimated at 1/100,000 births, with a female/male ratio 2:1.

P1.08 Hemolytic crisis induced by rasburicase administration revealing G-6-PD deficiency

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Mr BI, a 27-year-old man of Moroccan origin was hospitalized in our center for exploration of abdominal masses discovered by CT scan performed in the setting of gastric pain. Analysis of the surgical biopsy revealed the presence of a Burkitt's lymphoma (CD20+, CD5-, CD23-, CD22+, FMC7+, CD79b+, t(8;14)(q24;q32)). The Ann-Arbor stage was 4A. The blood analysis showed a complete blood count within normal range, an inflammatory

syndrome and cholestatic and cytolytic hepatitis. There was no cardiac, kidney or pancreatic impairment. Within 48 hours, debulking corticotherapy (methylprednisolone 20mg 3x/d IV) was started. To avoid tumor lysis syndrome, the patient received abundant hydration in association with rasburicase. Two days later, routine measurement of arterial saturation showed a SaO2 of 80% with 15% of methemoglobin and anemia (Hb 9.1g/dL). First asymptomatic (except for the macroscopic hematuria), the patient developed two hours later grade IV anemia (Hb 6.5g/dL) with evidence of hemolytic crisis (LDH: 5330 UI/L and bilirubinemia: 58.1 mg/L respectively). The patient was transferred to the intensive care unit for monitoring and received 6 units of red blood cells. The evolution was good with administration of the planned chemotherapy and achievement of a complete response.
A more detailed history of the patient revealed Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency in two brothers. This defect may induce, hemolytic anemia by decreasing the synthesis of NADPH, an essential enzyme that prevents aggression of the cytoplasmic membrane by free radical accumulation. Several drugs can induce this type of complication, including rasburicase, a recombinant urate oxidase.
Three months after the end of treatment, G-6-PD deficiency was confirmed by spectophotometric *measurement of the G-6-PD activity.*
This case report underlines the high incidence of G6PD deficiency in some ethnic groups and the importance of a detailed patient and family history before starting treatment even in case of emergency.

P1.09 Evaluation of automated white blood cell differential count in cerebrospinal fluid on the body fluid module of Sysmex XN 2000

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White blood cell (WBC) differential count in cerebrospinal fluid is an urgent and important analysis which is traditionally performed by microscopy on cytospin slides. This study investigated whether automated WBC differential count on Sysmex XN 2000 could serve as a suitable alternative for the manual method. Twenty-nine CSF samples with WBC count ≥ 10/µl were used for method comparison between the Sysmex XN 2000 and manual differentiation (200 WBCs) on cytospin slides. An excellent correlation without significant bias was found for the percentage (%) and absolute number (cells/µl) of polymorphonuclear cells ($y = 0.95x + 0.97$, $R^2 = 0.98$ and $y = 1.01x - 2.84$, $R^2 = 1.00$, respectively) and mononuclear cells ($y = 0.95x + 3.70$, $R^2=0.98$ and $y = 0.95x + 5.33$, $R^2 = 0.98$, respectively). Moreover, the XN 2000 agreed well with microscopic differentiation for the percentage and absolute number of neutrophils ($y = 0.95x + 0.92$, $R^2 = 0.98$ and $y = 1.00x - 2.38$, $R^2 = 1.00$, respectively) and lymphocytes ($y = 0.96x + 2.90$, $R^2 = 0.98$ and $y = 1.03x + 1.70$, $R^2 = 0.97$, respectively), but a small significant negative bias was found for monocytes ($y = 0.88x + 1.46$, $R^2 = 0.91$ and $y = 0.85x + 2.04$, $R^2 = 0.99$, respectively). In conclusion, this study found a good agreement between automated WBC differential count on Sysmex XN 2000 and manual differentiation. Therefore, this instrument is a fast and accurate alternative to microscopy for analyzing CSF.

P1.10 Immunomodulatory effects of Rapamycin in xenogeneic GVHD

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Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Several studies have shown that rapamycin (RAPA), an mTOR inhibitor with immunosuppressive properties, is an efficient treatment for many patients with GVHD, notably by favoring regulatory T cells (Tregs) proliferation in vivo and in vitro. However, very few data have been reported about the global impact of this drug on the immune system in the context of GVHD. The present work investigates the cellular mechanisms by which RAPA delays death from xenogeneic GVHD induced by human peripheral blood mononuclear cells infusion in NOD-scid IL-2Rγnull (NSG) mice. Our results show that RAPA injections significantly delay death from xenogeneic GVHD and reduce disease severity. Flow cytometric analyses highlighted a strong reduction of human cells chimerism in RAPA-treated mice in comparison to control mice, together with higher $CD4^+/CD8^+$ T cells balance due to a lower proliferation of $CD8^+$ T cells. In addition, the frequencies of naive $CD4^+$ and $CD8^+$ T cells were higher and the $CD4^+$ T cells showed a reduced effector phenotype ($CD45RO^+CD27^-$). Tregs were positively affected by RAPA that up-regulated their expression of Bcl-2 and Ki67 as well as their STAT5 phosphorylation level, leading to higher Treg frequency in treated mice. Altogether these data demonstrate that RAPA delays xenogeneic GVHD by lowering human chimerism and effector $CD4^+$ frequency as well as promoting Tregs.

P1.11 Acute myeloblastic leukemia infiltrating-T lymphocyte characterization, from bone marrow and peripheral blood

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Since a few years, there is increased evidence that the immune microenvironment plays a fundamental role in the outcome of leukemia. In particular, several groups have recently highlighted the role of regulatory T cells. In acute myeloblastic leukemia patients, we have assessed quantitatively and qualitatively the purified T cell population, using flow cytometry, Affymetrix microarray and quantitative RT-PCR array 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 leukemia as far as known prognostic factors are concerned. Patients clustering analyses revealed important significant differences between leukemic patients and healthy individuals in the gene expression profile of their T lymphocytes. 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, immunosuppressive molecules are expressed, T cell activation, inflammation and immune cell recruitment genes are expressed. We also observed significant differences in T cells from the different groups, showing that high risk AML patients have T cells that display a distinct genetic program than those from intermediate or "favorable"risk group, The same thing is observed when we perform an age super-vised analysis. However, a few differences were observed in remission compared to diagnosis in T cells.
Most of AML studies were focused on the leukemic blast biology,

but circulating immune cells in patients seem to reflect an important message. 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.

P1.12 Chronic EBV infection resulting in a CD20 negative lymphoproliferative disorder complicated with secondary hemophagocytosis

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A 27-year-old man presented with enlarged right axillar lymph nodes and progressive malaise, fever and night sweats. During the following months progressive weight loss and generalized lymphadenopathy developed. Peripheral blood analysis showed inflammation, microcytic anemia, normal platelet and white blood cell counts and elevated liver enzymes with normal LDH. Serology was compatible with chronic active EBV infection (IgM negative, IgG negative) with a viral load of $43x10^3$ copies/µg DNA. A lymph node excision biopsy was performed showing a strongly positive EBV staining. As evidence for a malignancy was lacking, the diagnosis of EBV related lymphoproliferative disease was withheld. Acyclovir and IV immunoglobulins initially resulted in a good clinical response but the EBV PCR remained positive and a few weeks later B-symptoms reappeared despite continued treatment. A new core needle biopsy of the largest PET positive axillary lymph node showed extensive geographical necrosis surrounded by an EBV positive CD20-/CD30+ blastic B-cell population, confirming now the presence of an EBV driven lymphoproliferative disorder. Because of progressive pancytopenia, a bone marrow analysis revealed a lymphoproliferative process and hemophagocytosis. The patient was treated with high dose corticosteroids and chemotherapy (CHOP). Etoposide was added in view of the hemophagocytosis. Using this strategy we obtained a good partial clinical response and the patient is still under active therapy. EBV PCR also became negative. As the patient had been EBV positive since several months and hemophagocytosis was present, an underlying immunodeficiency was investigated. HIV/AIDS, hypogammaglobulinemia/CVID, XMEN and XLP1 could be excluded. We are now looking into the presence of XLP2 through *XIAP* mutation analysis. We want to stress the importance to look for an underlying immunodeficiency in these patients because of the implications for further treatment and prognosis.

Posters lymphoid malignancies and mm P2.01-P2.23

P2.01 Downregulation of cellular microRNA-150 is a new prognostic factor associated with a poor prognosis in Chronic Lymphocytic Leukemia patients

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Background

MicroRNAs (miRs) are a new class of tumor suppressors frequently deregulated in cancer. Chronic lymphocytic leukemia (CLL) is characterized by a clinical heterogeneity that can be predicted by several prognostic factors. However, a better outcome individualization in a given patient is still of utmost interest.

Methods

We investigated the expression of microRNA-150 by real-time PCR (qPCR) from CD19+ cells or from CLL serums in a cohort of 273 CLL patients with a median follow-up of 78 months (range, 7-380) and correlated it to other biological or clinical parameters.

Results

We showed that miR-150 was significantly overexpressed in CLL cells (2.6 fold, P=0.0027) and CLL serum (4.6 fold, P<0.0001) compared to healthy subjects. Low cellular miR-150 expression levels was associated with tumor burden markers such as lymphocyte doubling time (P=0.0004) and soluble CD23 (P=0.0132), but also disease aggressiveness: cellular miR-150 level decreased significantly with progression from Binet Stage A to C (P<0.0149). In addition, low level of cellular miR-150 is found in poor prognostic subgroups defined by IgVH mutational status (P<0.0001), zêta-associated protein 70 (ZAP70) (P=0.0004), lipoprotein lipase (LPL) (P<0.0001), CD38 (P<0.0001) expression and cytogenetic abnormalities (P=0.0121). However, we did not find any correlation of serum miR-150 with all these markers. Cellular miR-150 was also associated with treatment-free (TFS) and overall survival (OS): patients with a low miR-150 expression have a median TFS/OS of 40/159 months compared to patient high level patient who have a median TFS/OS of 122/>380 months (P<0.0001/P=0.0129).

Conclusions

Downregulation of cellular miR-150 is associated with disease aggressiveness, tumor burden and poor clinical evolution. We concluded that cellular (but not serum) miR-150 level could be used as a new prognostic factor in CLL.

P2.02 The value of SOX11 overexpression in the differential diagnosis of mantle cell lymphoma

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Introduction

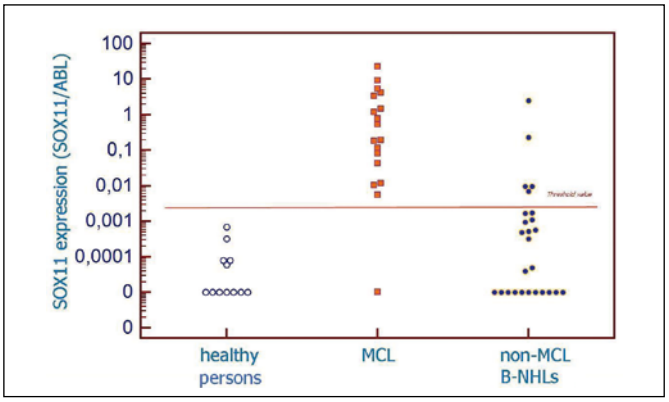
The diagnosis of mantle cell lymphoma (MCL) is multidisciplinary based on morphology, flowcytometry (CD5+/CD23-/CD200-) and *CCND1* rearrangement (demonstrated by cytogenetics or FISH). *CCND1* rearrangement or immunostaining is the hallmark of diagnosis. However, rare cases of *CCND1*- MCL have been described and would be associated with a poor clinical outcome and CCND2 or CCND3 overexpression. Recently, it is suggested that almost all MCL, *CCND1*+ or *CCND1*-, show overexpression of the SOX11 neural transcription factor. In this study, we determined the degree of SOX11 expression and evaluated its value as a marker in the differential diagnosis of MCL.

Material and methods

RNA was extracted from blood, bone marrow or lymph node samples, followed by DNase treatment (Ambion®Turbo DNA-free™) and cDNA synthesis. TAQMAN gene expression assays for SOX11 and *CCND1* expression were used according to manufacturer's instructions (LifeTechnologies). Three sample cohorts consisting out of 18 C*CND1*+ MCL (confirmed by FISH analysis, clonal lymphocytes ranging between 5%-93% as determined by flowcytometry), 26 non-MCL B-NHLs (14 CLL, 2 atypical CLL, 4 SLVL, 2 HCL, 2 B-NHL NOS, 1 LPL and 1 MZL) and 12 healthy persons were analyzed.

Results
A SOX11 overexpression threshold was defined based on the results of healthy persons and *CCND1*+ MCL. Surprisingly, we observed that 4/26 non-MCL B-NHLs (2 typical CLL, 1 atypical CLL and 1 SLVL) showed overexpression. Only 1/18 *CCND1*+ MCL did not show SOX11 overexpression.

Conclusion
Absence of SOX11 overexpression excludes MCL with a very high probability (NPV 96%, sensitivity 94%). However, SOX11 overexpression is not specific for MCL and can be seen in other B-NHLs (PPV 81%, specificity 85%). The assay can thus be useful in the differential diagnosis of MCL eg if *CCND1* rearrangement results are not available or if a quick response is required.



P2.02 *Figure 1*

P2.03 Immunomodulatory Drugs Restore Effector Cell Immune Functions In Myeloma Patients With Low Disease Burden After Autologous Stem Cell Transplantation

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The micro-environment in multiple myeloma is highly immunosuppressive with increased numbers of regulatory T cells and myeloid derived suppressor cells favoring tumor cell survival and hampering immunotherapeutic strategies such as dendritic cell vaccination. Immunomodulatory drugs are known to enhance T- and NK-cell function. In this study we evaluated the *ex vivo* effects of low dose lenalidomide and pomalidomide on the functionality of $CD8^+$ and $CD4^+$ T cells, regulatory T cells, myeloid derived suppressor cells and *ex-vivo* generated mononuclear derived dendritic cells obtained from MM patients after first autologous stem cell transplantation with low residual disease burden. We observed that lenalidomide and pomalidomide increase $CD4^+$ and $CD8^+$ T-cell proliferation, enhance cytokine production and reduce the suppressive effects of regulatory T cells and myeloid derived suppressor cells on $CD8^+$ T cell responses. The effects of pomalidomide were generally more profound compared to lenalidomide. In addition, we found that functional dendritic cells can be generated from mononuclear cells obtained by leukapheresis form these multiple myeloma patients and that the presence of immunomodulatory drugs enhanced dendritic cell-induced T-cell responses characterized by a higher degree of polyfunctionality, i.e. the capacity to produce several types of cytokines on a single cell basis. These results provide a preclinical rationale for the design of early phase clinical studies to assess safety and efficacy of dendritic cell-based immunotherapy in combination with immunomodulatory drugs in multiple myeloma patients.

P2.04 Immune impairments of the multiple myeloma bone-marrow mesenchymal stromal cells

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In multiple myeloma (MM), bone marrow mesenchymal stromal cells (BM-MSC) play an important role in pathogenesis and disease progression by supporting myeloma cell growth and by inducing drug resistance. Previous studies have suggested that direct and indirect interactions between malignant cells and BM-MSC result in constitutive abnormalities in the MM BM-MSC. The aim of this study was to investigate the mechanisms involved in the abnormal immunomodulation capacities of MM BM-MSC. We analyzed the MM BM-MSC expression of diverse adhesion molecules and immune effectors in constitutive and inflammatory conditions in comparison with MSC from healthy donors (HD BM-MSC). We measured the concentration of immunoregulatory cytokines in co-cultures between activated T cells and MM BM-MSC. Finally, we evaluated the fate of activated T cells co-cultured with MM BM-MSC. We demonstrated that MM BM-MSC have an abnormal expression of CD40/40L, VCAM1, ICAM-1, LFA-3, HLA-DR and HLA-ABC. We observed an overproduction of IL-6 and a reduced secretion of IL-10 when MM BM-MSC were co-cultured with activated T lymphocytes compared to HD BM-MSC. An increased Th17/Treg ratio was observed when activated T cells were co-cultured with MM BM-MSC compared to HD BM-MSC. Our observations demonstrated that MM BM-MSC altered immunomodulation capacities were linked to variations in their immunogenicity and secretion profile. These alterations lead to a reduced inhibition of T cell proliferation but also in a switch of the Th17/Treg balance. We identified several potential factors responsible of these alterations such as IL-6, VCAM-1 and CD40/40L that could also be associated to MM pathogenesis and progression.

P2.05 Global HDAC enzymatic activity is a strong marker of poor prognosis in Chronic Lymphocytic Leukemia

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Background
Histone deacetylases (HDACs) play a crucial role in transcriptional regulation by modulating chromatin structure. We recently demonstrated that most of HDAC isoenzyme mRNA was deregulated in chronic lymphocytic leukemia (CLL) compared with normal B cells (Van Damme et al, Epigenetics 2012). However, global HDAC enzymatic activity has not been yet investigated in CLL B-cells in terms of expression and prognosis.

Methods
A cohort of 114 patients with a median follow-up of 91 months (range, 11-376) was investigated in this study. Protein extracts were prepared from CD19+ purified CLL cells (obtained at diagnosis) and compared with normal B-cells. Deacetylation activity, expressed as pmol of deacetylated substrate normalized by GAPDH expression, was correlated with classical prognostic factors (IgVH, Binet stage, ZAP70, LPL and CD38) and clinical data.

Results
Global HDAC activity of leukemic B-cells was not significantly

different from normal B cells (P>0.05). When we stratified patients according to low (n=75) and high (n=39) global HDAC activity (binarized using ROC curve analysis), we observed that this value was significantly correlated with treatment-free (TFS, P=0.0156) and overall survival (OS, P<0.0001): patients with a low HDAC activity have a median TFS and OS of 101 and >376 months respectively while patients with a high HDAC activity have a median TFS of 47 months and a median OS of 137 months. Multivariate Cox regression analysis indicated that HDAC activity is a significant independent prognostic factor for OS prediction (Hazard Ratio=4.13; P=0.0013) able to refine classical prognostic factors as IgVH, Binet stage, ZAP70, LPL and CD38. Finally, HDAC activity is increased after B-cell receptor stimulation using IgM suggesting the potential role of microenvironment stimuli (P = 0.0313, n=6).

Conclusions
Global HDAC activity is a prognostic factor for TFS and OS prediction and an independent marker for OS in multivariate analysis which allows us to refine other prognostic factors. This work provides a rational biological base for HDAC inhibitor use and strongly supports their clinical application.

P2.06 Diagnosis of lymphoma in a patient under treatment with azacytidine

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We report on a 72y old male patient, with a history of smoking, COPD and ischemic cardiac disease with a CABG at the age of 60. He was referred to the hematology department with pancytopenia. A bone marrow biopsy and aspirate showed a myelodysplastic syndrome with trilineage dysplasia (MDS-RCMD) with a central blastosis of 11% and a complex karyotype. He was considered ineligible for transplantation and started on a treatment with azacytidine. The peripheral blood counts normalized after 3 cycles. After the 10th cycle, a pneumonia required hospitalization. After 12 cycles of azacytidine, a new bone marrow aspirate showed a decrease of the central blastosis to 5% and the patient continued his treatment with azacytidine. After 14 cycles, the peripheral blast count increased above 10% and the patient developed a difficulty swallowing, due to an 8 cm large cervical lymph node. Biopsy of the node revealed a diffuse large B-cell lymphoma, germinal center type. Staging with PET-CT confirmed this was a solitary lesion. Azacytidine was stopped and one course of mini-R-CHOP was administered. Eleven days after chemotherapy, the patient was admitted with febrile neutropenia, pneumonia and oral herpes. The treatment was modified to rituximab plus local radiotherapy. The patient wished to stop the radiotherapy after 9 of 18 planned sessions. Three weeks later, he was hospitalized with evolution to acute myeloid leukemia with 76% peripheral blasts, pneumonia and severe cachexia. After a short course of IV antibiotics, the patient died within 24hours after the institution of a palliative treatment.

The course of the patient, who died of AML, 17 months after the diagnosis of MDS-RCMD IPSS-2 is not unusual. However, the diagnosis of lymphoma, while under treatment with azacytidine, is intriguing. One hypothesis is that epigenetic modification could stimulate lymphomagenesis. However, the absence of reports on the combination of MDS, treated with azacytine, and lymphoma makes this hypothesis questionable. More likely, the DLBCL was a manifestation of increased immune deficiency, due to disease progression.

P2.07 Bing-Neel syndrome: report of two cases

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Introduction
Waldenström macroglobulinemia (WM) is defined as a B-cell neoplasm with the morphological presence of small B-lymphocytes, plasmacytoid lymphocytes, plasmacells and an IgM paraprotein. Infiltration of the central nervous system, referred to as the Bing-Neel syndrome, is a rare and usually late complication of the disease. It should be distinguished from the more frequent neurological complications such as hyperviscosity syndrome and peripheral neuropathy.

Case presentation
A 65-year old woman and 82-year old man, both with a long-standing history of WM, presented with neurological symptoms (headache, nausea, vomiting, vertigo and blurred vision). The first case was initially misdiagnosed and treated for an iatrogenic meningitis. However, neurological symptoms persisted and further investigations were needed. In the second case ischemic stroke was clinically suspected but imaging could not confirm this.

Results
Laboratory work-up of the first patient showed anemia (Hb 10.2 g/dL) and a serum monoclonal IgM paraprotein (11.7 g/L). The second patient presented with comparable values (Hb 10.0 g/dL, IgM paraprotein 26.5 g/L). Lumbar punction in both patients revealed an increased white blood cell count (59/mm^3 and 30/mm^3, respectively). Microscopic assessment showed the presence of plasmacytoid lymphocytes and plasmocytes. Immunophenotyping revealed in both cases the presence of a monoclonal B-cell population (CD5-CD10-CD20+CD23-CD79b+FMC7+IgM+), compatible with the WM phenotype. In the first case the presence of IgM lambda paraprotein in the cerebrospinal fluid was confirmed by immunofixation and a clonal pattern for IgH FR3JH, IgKb, and IgKa by molecular analysis. In the second case IgM kappa paraprotein was detected and a clonal pattern for IgH FR1JH, IgH FR3JH and IgKa. Both patients were treated with intrathecal methotrexate, dexamethasone and cytarabine and the first case also with whole brain radiotherapy. The prognosis of this syndrome is generally poor. Also in these cases it was decided to switch to palliative treatment 6 respectively 4 months after the diagnosis.

Conclusion
In the follow-up of patients with WM, a multidisciplinary work-up is indispensible for an accurate diagnosis of the Bing-Neel syndrome when patients present with neurological symptoms.

P2.08 Effect of different peak integration methods on the accuracy of paraprotein quantification on serum protein electrophoresis: what is the clinical relevance?

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The quantification of a paraprotein in serum is performed by protein electrophoresis. Two different peak integration methods can be used: the perpendicular drop method and the tangent skimming method. To assess the differences between these two methods, 59 serum samples containing a quantifiable (≥ 5%) IgG paraprotein with a migration in the γ-region were analysed using capillary electrophoresis on Capillarys II (Sebia). The two

integration methods were also correlated to quantitative IgG Kappa and IgG Lambda (Hevylite) determinations.
All paraprotein concentrations obtained with the perpendicular drop method were higher than the tangent skimming method. The average difference between the two integration methods was 53% (3 g/l). The maximum relative difference between the two methods was 137%, but the absolute difference was maximally 5.4 g/l. The largest relative differences were seen in paraprotein levels lower than 15 g/l. Comparable results were seen with the Hevylite measurements: the differences between the two integration methods were smaller in samples with a very high or very low IgG K / IgG L ratio.
These data and previously published data, show that the quantitation of low paraprotein concentrations (<15 g/l) is inaccurate. The most correct way to estimate the 'real' paraprotein concentration is probably through the use of the tangent skimming method. The perpendicular drop method which is used in most clinical laboratories probably overestimates the paraprotein concentration. For prognostic evaluation of the patient, the relevance of this inaccuracy on a single sample measurement should however be considered less relevant. Also, probably most scientific studies, and subsequent guidelines on monoclonal gammopathy and related syndromes, are based on the perpendicular drop method. Nevertheless, it should be stressed that the follow-up of patients harbouring a paraprotein, especially with low levels (< 15 g/l), should not only be done by using the same method but also in the same laboratory to ensure that the paraprotein estimation is performed in a similar fashion.

P2.09 Impact of minimal residual disease monitoring on therapy in Belgian childhood acute lymphoblastic leukaemia

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In Belgium, each year approximately 70 children are diagnosed with acute lymphoblastic leukaemia (ALL). For these children, the monitoring of minimal residual disease (MRD) by immunoglobulin and T cell receptor PCR has an important prognostic value and is used to stratify the patients into different risk groups and to adapt therapy to overcome undertreatment or overtreatment. The risk group stratification and treatment protocols of Belgian children with ALL are based on the EORTC-CLG guidelines. According to these guidelines, the MRD monitoring is performed after induction therapy (time point 1A) and after consolidation therapy (time point 1B).
Children at low or intermediate risk with MRD $\geq 10^{-2}$ at time point 1A are switched to a high risk treatment protocol. This occurred in four of the 141 Belgian patients (3%) at low or intermediate risk between 2010-2012.
At time point 1B, only high risk patients with MRD $\geq 10^{-3}$ will be prepared for allogeneic stem cell transplantation (SCT). Between 2010 and 2012, SCT could be avoided in 14 of the 27 high risk patients (51%) due to MRD $<10^{-3}$.
In relapsed patients, MRD monitoring at the end of reinduction therapy is an important risk factor. Between 2010 and 2012, SCT could be avoided in 8 of the 24 relapsed patients (30%) based on MRD results.
In conclusion, MRD monitoring by Ig/TCR PCR in childhood ALL is a valuable tool to adapt therapy. Based on MRD data, children can be switched to a more intensive treatment to avoid a possible relapse. On the other hand, a less intensive treatment can be given in a considerable number of newly diagnosed and relapsed patients without impairing their outcome.

P2.10 BRAF V600E mutation is not a disease-defining genetic event in classic hairy cell leukemia

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Hairy cell leukemia (HCL) is uniquely sensitive to chemotherapy with overall survival rates exceeding 90%. Initial reports described a virtually 100% diagnostic sensitivity and specificity of the BRAF V600E mutation for HCL in hematological malignancies (1). Xi et al. recently found a lack of BRAF V600E mutation in 11 of 53 classic HCLs and reported that all IGHV4-34 expressing HCLs lack the BRAF V600E mutation (2). IGHV4-34 expressing HCLs show a shorter overall survival due to resistence to chemotherapy (3).
We developed a sensitive real-time PCR with a blocking locked nucleic acid primer for detection of the BRAF V600E mutation. A reproducible sensitivity of 1% tumor DNA in normal DNA was reached. IgH rearrangement sequencing was performed using 6 IgHV FR1 primers combined with one IGHJ consensus primer as designed by the Biomed-2 study, followed by Sanger sequencing. VH gene usage was determined only for productive rearrangements. Bone marrow or peripheral blood samples taken at diagnosis from 22 HCL patients were analysed. Morphologic and flowcytometric analysis of all samples confirmed the diagnosis of classic HCL based on WHO classification.
The BRAF V600E mutation was detected in 19 of 22 (86%) patients. VH gene usage data was obtained for 14 patients. One functional VH4-34+ rearrangement was observed, indeed coinciding with a wild type BRAF HCL. This patient showed normal response to cladribine chemotherapy. For the other two wild type BRAF HCL cases respectively a VH1-2 and VH3-11 functional rearrangement was detected.
Due to the high prevalence of the BRAF V600E mutation in HCL, it's sensitive detection is useful for diagnosis of HCL, however not to be considered as a disease-defining marker. More cases of HCL should be studied to correlate BRAF mutation status and VH4-34 gene usage with refractoriness to initial chemotherapy.
1) Tiacci, NEJM 2011: 2305; Boyd, BJH 2011:609; Arciani, Blood 2012: 188
2) Xi, Blood 2012:3330
3) Arons, Blood 2009: 4687

P2.11 Activating JAK1 and JAK3 mutations cooperate to confer growth signal self-sufficiency and increased resistance to JAK inhibitors in a mouse in vitro leukemia model

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The acquisition of growth signal self-sufficiency is one of the hallmarks of cancer cells. We previously reported that the murine IL-9-dependent TS1 cell line can give rise to autonomous clones with constitutive activation of the JAK-STAT pathway. Here, we show that this transforming event results from acquisition of activating mutations either in JAK1, in JAK3 or in both kinases. Interestingly, similar mutations, localized in the kinase or pseudo-kinase domains of these JAKs are observed in patients with ALL or NK/T cell lymphomas, some of which also show mutations in both JAK1 and JAK3. Transient and stable expression of JAK1 and/or JAK3 mutants showed that each mutant alone can induce STAT5 activation, and that their coexpression further increases this activation. This cooperation was confirmed in a dexamethasone-sensitive T cell lymphoma model, in which the JAK-STAT pathway confers resistance to corticoid-induced cell death.
The proliferation of TS1 autonomous clones can be efficiently blocked by JAK tyrosine kinase inhibitors such as ruxolitinib or CMP6 in short term assays. However, resistant clones occur at low frequency upon long term culture in the presence of kinase inhibitors. This process is reminiscent of the acquisition of imatinib-resistance in BCR-Abl-transformed cells, but the underlying mechanism turned out to be distinct. Indeed, this resistance was not caused by the acquisition of secondary mutations in the ATP-binding pocket of the JAK mutant. Surprisingly, cells that originally showed a JAK1-activating mutation became resistant to inhibitors by acquiring another activating mutation in JAK3, whereas, vice versa, cells that originally showed a JAK3-activating mutation became resistant to inhibitors by acquiring another activating mutation in JAK1. These observations further underline the cooperation between JAK1 and JAK3 activation in T cell transformation, and represent a new mechanism of acquisition of resistance against JAK kinase inhibitors.

P2.12 An unusual presentation of cryptococcal meningitis in a patient with lymphoplasmacytic dyscrasia

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A 62-year-old Caucasian male with lymphoplasmacytic B-cell non Hodgkin lymphoma, was referred for confusion without fever since 3 weeks. He reported a pulmonary infection treated with amoxicillin-clavulanate 1 month earlier. His previous treatments included fludarabine, high-dose chemotherapy (BEAM) followed by autolog-ous stem cell transplantation (2000), and by plasmaphereses and chemotherapy with fludarabine, cyclophosphamide and Rituximab 14 months earlier (4 cycles). Neurologically, physical examination was normal.
Routine laboratory evaluation was normal except for a raised immunoglobulin M (IgM)(3490 mg/dl). Initially assuming that the confusion was secondary to hyperviscosity induced by the increased IgM, the patient underwent plasmaphereses.
Given the persistence of symptoms, a magnetic resonance imaging (MRI) of the brain was performed and showed enhancement of pia mater and ventricular ependyma. Cerebrospinal fluid (CSF) examination disclosed 68 cells/mm³ (91% of neutrophils), low glucose (8 mg/dl) and elevated protein levels (1111 mg/dl). Unless the India ink examination of the CSF was negative, culture was positive for Cryptococcus neoformans, and cryptococcal antigen titer was 1/32 in the CSF. CD4+cell count was 74/mm³. Therapy with amphotericin B and fluconazole was initiated on day 12 after admission.
After one week of combined therapy, neurological symptoms worsened. A new MRI showed hydrocephalus, increased signs of meningoencephalitis and the emergence of cerebellitis. To lower intracranial pressure, a ventricular-peritoneal drain was inserted. It was hypothesized that neurological deterioration was due to an immune reconstitution inflammatory syndrome (IRS). After 5 weeks of continuous therapy, cryptococcal antigen and mycotic culture of the CSF became negative, but raised protein concentration remained present as well as pleocytosis. The patient died in the context of overt rhombencephalitis. The present report illustrates that cryptococcosis still represents a devastating opportunistic infection in the immunocompromised patient, more specifically with T-cell immunodeficiency. This infection should be always considered in patients with neurological symptoms and lympho-proliferative disease previously treated with chemotherapy. Post Fludarabine CD4+ cell counts are often low and persist for periods lasting for 12-24 months.

P2.13 Management of refractory/relapsed diffuse large B-cell lymphoma (DLBCL): the gap between guidelines and real world

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Background
During the last decade, first-line regimens associating rituximab to chemotherapy (CT) has both improved remission rates and survival of patients with DLBCL; nevertheless, one-third of patients have disease that is either refractory or relapsed (R/R) after standard therapy. According to currently available guidelines, salvage CT followed by high dose therapy (HDT) and autologous stem-cell transplantation (ASCT) is considered the standard treatment for R/R patients with chemotherapy-sensitive disease. So far, reproducibility of guidelines to clinical practice has never been investigated.

Patients and methods
We have conducted a population-based study on all cases of DLBCL collected from Modena Cancer Registry, in northern Italy from 1997 to 2011. For the purposes of this study we identified patients who were eligible to HDT followed by ASCT using the following criteria: use of conventional CT as induction treatment, progressive disease after induction therapy or relapse during follow-up. Patients were also analyzed by age group. The study endpoint was the rate of transplanted patients. The aim of the study was to compare the observed transplant rate (TR) to the theoretical TR and to describe patients outcome.

Results
Overall, 850 cases of DLBCL were initially identified for this study, 640 of them received CT and 272 relapsed/progressed during follow-up. Salvage therapy could be administered in 147 patients (54%) and included HDT-ASCT in 16 cases (TR=11%). Looking at patients younger than 65 years, salvage therapy was administered in 69 out of 101 R/R cases and consisted of HDT-ASCT in 12 cases (TR=17%). In 44 (30%) and 24 (35%) additional patients in the overall study population and in the younger subgroup, respectively, intensified CT regimens were used (including ICE, DHAP and GDP) not followed by ASCT, due lack of response.

Conclusion
Although HDT-ASCT is suggested as standard therapy for R/R DLBCL, this strategy is actually adopted in a small proportion of cases, mainly due to lack of response to salvage treatment. Strategies to improve anti-lymphoma activity of salvage therapies are warranted.

P2.14 Cost-utility of granulocyte-colony stimulating factors (G-CSFS) for primary prophylaxis (PP) of chemotherapy induced febrile neutropenia (FN) in non-Hodgkin's lymphoma patients (NHL) in Belgium

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Objective
Assess the cost-utility in Belgium of PP with once-per-cycle pegfilgrastim vs. no prophylaxis, vs. PP with daily filgrastim or lenograstim (11-days as per label or 6-days suboptimal use) and vs. secondary prophylaxis (SP) with pegfilgrastim; for reducing FN incidence in patients with NHL receiving standard (R)-CHOP. Additionally, the number needed to treat (NNT) to avoid an FN episode is estimated for the different PP options vs. no prophylaxis.

Methods
A decision-analytic model was constructed from the healthcare-payer perspective. Costs were obtained from official list prices (Oct 2013) or literature and included drugs, drug administration and FN-related hospitalisation costs. Effectiveness inputs were based on a recent meta-analysis and outcomes in previous NHL studies. Survival and utility inputs were modeled from available data for NHL patients in the US. Outcomes included NNT and incremental cost per quality-adjusted life-year (QALY) gained. Univariate sensitivity analyses evaluated the robustness of the model.

Results
NNT in PP was lowest with pegfilgrastim at 4.7, with 11-days filgrastim at 8.5 and 6-days filgrastim at 15.5 In terms of incremental cost-utility ratio (ICUR), PP pegfilgrastim was dominant vs. PP 11-days lenograstim and was considered cost-effective vs. no prophylaxis (€ 20,644/QALY); vs. PP 11-days filgrastim (€ 3,210/QALY) and vs. PP 6-days filgrastim and lenograstim (€ 14,567 and € 8,218 per QALY respectively) as well as vs. SP pegfilgrastim (€24,759/QALY). The sensitivity analyses revealed that the most sensitive variables were G-CSF effectiveness, incremental survival assumptions and the cost of FN related hospitalisation, and that the model was robust. In a scenario analysis reducing the prices of daily G-CSFs by 50%, PP pegfilgrastim remained cost-effective, with ICURs below a threshold of € 30,000/QALY vs. all daily G-CSF options.

Conclusion
In a Belgian health care setting,PP pegfilgrastim is a cost-effective approach to FN in NHL patients at high risk for FN. In the cost-utility analysis PP pegfilgrastim was dominant vs. PP 11-days lenograstim and cost-effective vs. no prophylaxis and vs. all other FN prophylaxis strategies.

P2.15 Flow cytometry characterization of micro-particles from healthy and malignant blood cells

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Introduction
Recently, microparticles (MPs) have been described as a new way of intercellular communication. MPs are plasma membrane fragments with sizes ranging from 0.1 to 1µm, containing products of the original cell, such as microRNA, mRNA and proteins that can be delivered to other cells. However, little is known about their phenotypic characterization.

Material and methods
We analyzed MPs from platelets, B-cells, T-cells, NK-cells, monocytes, mesenchymal stromal cells (MSC) and chronic lymphocytic leukemia (CLL) B-cells. Cells were purified by positive magnetic-separation and cultured during 48h. Cells and MPs (obtained after ultracentrifugation at 20000g) were phenotypically characterized by the following monoclonal antibodies (CD19,20,184,45 for B-cells, CD3,8,5,27 for T-cells, CD16,56 for NK-cells, CD14,11c for monocytes, CD73,90,146,HLA for MSC, CD41,61 for platelets). Isolated MPs were stained with annexin-V-FITC and gated between 300nm and 900nm. Then latex bead technique for easy detection of MPs (increasing element's size and antigen's detection) was also performed.

Results
For all samples, MPs defined as positive event for annexin-V and included in gate of 300-900nm of size were detected. MPs production was confirmed by electronic microscopy. In principle, MPs are characterized by antigen expression from mother cells. Our results showed that characteristic antigens of platelet (CD41 and CD61) were found on platelet-derived MPs, however, for other cell type-derived MPs; we were not able to detect any antigen present on the original cells (T, B, NK, monocytes, MSC or CLL-cells) while these antigens were clearly expressed on the cells. Using latex bead technique, we confirmed detection of CD41 and CD61 on platelet-derived MPs. However, for all other antigens, results found turned out to be false positives, proved by the use of other type of negative controls (same labeling on MPs from different origins).

Conclusion
Here, we observed that mother cell antigens were not always detected on corresponding MPs by flow cytometry or latex beads. Our results demonstrated that the characterization of the MPs is a difficult field requiring the use of several negative controls.

P2.16 Activation of invariant natural killer T cells reduces angiogenesis in the 5T33 multiple myeloma model

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Angiogenesis refers to the generation of new vasculature from pre-existing blood vessels. In cancer, it is required for tumor growth. We used the immunocompetent 5T33MM model to investigate the anti-angiogenic effect of iNKTs. iNKTs respond to glycolipids such as α-Galactosylceramide (α-GalCer). We first examined whether α-GalCer-activated iNKTs have an effect on tumor angiogenesis by treating 5T33MM mice with α-GalCer. A significant decrease of the microvessel density (MVD) was observed in the treated group (23.7%) compared to untreated group (33.3%). This was independent of the reduction in tumor load indicating that the secretory products of iNKTs such as IFN-γ can reduce angiogenesis. To proceed further, conditioned media (CM) were prepared by coculturing DC with iNKT in presence (CM^+) or absence (CM^-) of α-GalCer. Chorioallantoic membrane (CAM) and matrigel plug (MP) assays were performed by injecting CM into fertilized chicken eggs and NOD-SCID mice, respectively. Results showed a reduction in both blood vessel count in CAM (from 26 in CM^- to 13 in CM^+) and $CD31^+$ cell count in MP (from 112 in CM^- to

25 in CM+). To see whether this inhibition is mediated by IFN-γ, endothelial cells (ECs) were cultured in vitro in CM, IFN-γ and CM+α-IFN-γ antibody. Cell Titer-Glo assays showed a significant reduction in the viability of ECs in CM+ and IFN-γ. This reduction was partially blocked when CM+ was blocked by α-IFN-γ. To determine if the cells' low viability is related to anti-proliferative and/or apoptotic mechanisms, proliferation and TUNEL assays were performed. We found a reduction in ECs proliferation in CM+. This defect was confirmed using matrigel capillarogenesis assay which was then used for TUNEL stainings. A high number of apoptotic cells was observed in CM+ (18%) compared to CM- (7%) and control (8%). Therefore, we can conclude that anti-angiogenesis can be achieved by both pathways, and IFN-γ is involved as an angiostatic factor. Taken together, these preclinical data indicate the possibility of harnessing the anti-angiogenic activity of iNKTs in MM.

P2.17 The respective value of the G8 screening and the Comprehensive Geriatric Assessment (CGA) for the identification of vulnerable older patients with hematological malignancies susceptible to benefit from chemotherapy

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Background

Using a Comprehensive Geriatric Assessment (CGA) is recommended in order to detect vulnerable cancer patients for whom chemotherapy can have severe potential impact on functionality, quality of life, life-threatening toxicity or survival. The G8 screening tool described for older cancer patients requires validation in older patients with hematological malignancies susceptible to benefit from chemotherapy.

Aims

To assess in older patients with hematological malignancies susceptible to benefit from chemotherapy the reliability of the G8 screening tool compared to a full CGA. To assess in patients with malignant hemopathies the predictive value of G8 and CGA in terms of overall survival.

Methods

G8 and CGA were proposed to 107 consecutive patients (65-89yrs) with hematological malignancies admitted to receive chemotherapy. An initial full-dose or reduced-dose chemotherapy has been administrated to patients according to a multidisciplinary team decision.

Results

Ninety patients were evaluable for both scales, of which 72% and 80%, were defined as "vulnerable"when evaluated with G8 (≤14.5) or CGA (≥2 impairments), respectively. The area under ROC-curve of G8 compared to CGA was 0.749 ± 0.051. A sensitivity of 79.2% and a specificity of 55.6% for G8 were obtained. Neither the G8 nor the CGA were associated with the initial treatment choice or predictive for one-year survival in our series. Noteworthy, some specific items of the CGA such as renal insufficiency (p=0.023), cognitive impairment (MoCA) (p=0.030) and falls (p=0.013) were more useful to predict survival.

Conclusions

In our small series of older patients with hematological malignancies, G8 has a moderate performance to identify patients who should benefit from a CGA. G8 is not correlated with initial treatment choice and does not seem to predict survival. However, renal insufficiency, cognitive impairment and falls are more useful to predict survival. Prospective trials are thus needed to further determine whether G8 combined with these three specific items could be more adapted to older patients with hematological malignancies to identify a "fit"population requiring full dose chemotherapy.

P2.18 Responding patients show durable responses to bendamustine in double refractory multiple myeloma patients

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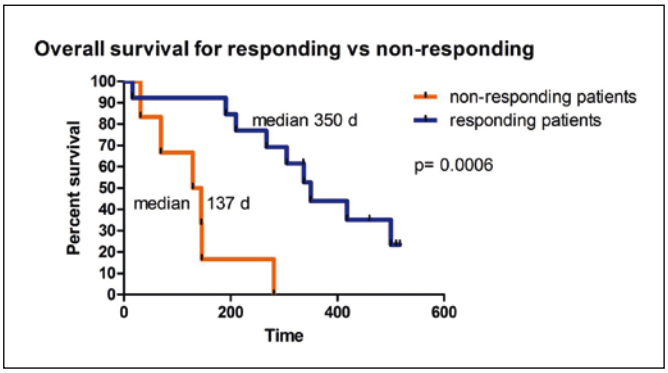
Bendamustine - an alkylating agent with purine-analogue like activities - shows activity in both de novo and relapsed/refractory MM patients. The BHS MM study group initiated a study from February till December 2012 and included 20 patients with a prior history of relapse after both bortezomib and lenalidomide treatment. Other inclusion criteria were the absence of end-stage renal disease, a correct residual marrow function and the absence of plasma cell leukemia.

Twenty patients with a median age of 69 years (52y-83y) received bendamustine until disease progression. The median number of bendamustine cycles infused was 4 (1-8). Patients were *heavily pretreated* (*mean number* of prior regimens was 5, *ranging from 3 to 8*). Eleven patients received at least one autologous stem cell transplantation. Responses were assessed by the treating physician. The overall response rate (according to EBMT criteria) was 45% (1 very good partial response and 8 partial responses), 2 patients showed a minor response, 3 patients a stable disease and disease progression was described in 6 patients. The progression free survival (PFS) for the whole population was 90 days, but responding patients had a PFS of 133 days (compared to 60 days in non-responding patients, p= 0.001). Overall survival was 350 days for responding patients compared to 137 days for non-responding patients (p=0.0006). The toxicity was mainly haematological with gr III-IV cytopenia in 50% of patients. One patient presented a septicaemia and another one a CMV infection.



P2.18 *Figure 1*

In this heavily pretreated patient population, survival rates for patients responding to bendamustine salvage therapy were encouraging. We believe that well-selected double refractory patients might benefit from bendamustine as salvage treatment, but further prospective clinical trials are needed in this situation.

P2.19 Light chain cast nephropathy in a patient with Waldenström's macroglobulinemia

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A 65-year-old man, diagnosed with Waldenström's macroglobulinemia (WM), who had repeatedly declined systemic treatment presented with anuric renal failure and hyperviscosity syndrome (HVS). Viscosity was increased to 3.2 and immunoglobulin M-levels (IgM) to 54.80g/L. Plasma exchange was initiated to relieve HVS-symptoms. At presentation plasma creatinin had increased from 1.32 mg/dl to 7.16 mg/dl and eGFR had decreased from 54ml/min/1.73m2 to 8 ml/min/1.73m2. Urgent haemodialysis was started, dexamethason was also administered, however renal function did not improve. Kidney biopsy revealed protein sediments in the tubules, kappa positive on immunofluorescence, compatible with light chain cast-nephropathy. Serum free light chain kappa was elevated (1120 mg/L), a repeat bone marrow aspirate confirmed the diagnosis of WM. Since there was a light chain excess and a biopsy proven light chain cast nephropathy haemodiafiltration with endogenous reinfusion (HFR) was initiated and continued for the first time in a patient with WM. During diagnostic work-up dexamethasone was started, however renal function did not improve. Subsequently systemic treatment was initiated with R-COP (rituximab, cyclophosphamide, vincristine and prednisolone), in accordance to a previous report. There was a sufficient improvement in renal function to stop haemodialysis, unfortunately the patient progressed after 3 treatment cycles and deceased of splenic rupture.
WM is a rare low-grade lymphoproliferative disorder, histopathologically defined as a subset of lymphoplasmacytic lymphoma (LPL). WM is characterized by bone marrow infiltration and clonal proliferation of B-lymphocytes responsible for IgM monoclonal gammopathy of any concentration. Most patients diagnosed with WM have symptoms attributable to tumor infiltration and/or monoclonal protein. Renal disease in WM has been reported, but it is rare and cast nephropathy associated with WM in particular is very rare. To our knowledge this is the sixth case report of WM-associated cast-nephropathy.

P2.20 Galectin expression in the multiple myeloma microenvironment

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Galectins are a protein family characterized by a conserved carbohydrate recognition domain, which enables them to bind to and cross-link glycoproteins. In addition, they exert intracellular functions independent of their sugar-binding capacity. Galectins have been implicated in diverse physiological and pathological processes, including cancer. In fact, galectins have been shown to contribute to multiple aspects of tumorigenesis, including neoplastic transformation, tumor cell survival, immune escape, angiogenesis and metastasis. This makes these proteins interesting targets for therapy. At this point, the role of galectins in multiple myeloma is not well understood. Interestingly, initial reports suggest a similar multi-faceted role for galectins in this disease.
Multiple myeloma is a hematological malignancy of monoclonal plasma cells characterized by bone marrow infiltration of these cells and osteolytic bone lesions. Galectins have been shown to modulate the proliferation and survival of multiple myeloma cells and the differentiation, proliferation and adhesion of osteoblasts and osteoclasts. In the current project, we aim to elucidate the role of galectins in the 5TGM.1 model with a focus on their role in osteolytic bone disease. We profiled the mRNA expression of all murine galectin family members in 5TGM.1 myeloma cells before and after propagation *in vivo*. In addition, we established primary osteoclast cultures and we confirmed successful generation of polykaryotic osteoclasts by TRAP staining and reporter gene expression validation. Also, we determined galectin mRNA expression during osteoclast differentiation from monocytic precursors. Our results indicate that the expression of several galectins is significantly altered during these processes.
These initial results provide us with target galectins for gain- and loss-of function experiments to further dissect their contribution to multiple myeloma biology and the development of osteolytic bone disease.

P2.21 Post-Transplant Lymphoproliferative Disorder following solid organ transplantation

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Background
Post-Transplant Lymphoproliferative Disorder (PTLD) is one of the most frequent cancer following solid organ transplantation. Its incidence has been noted to vary according to EBV history and time from transplant.

Population and methods
We reviewed all cases of patients which had developed PTLD after solid organ transplantation (heart, lung, kidney and liver) from 1987 to 2010, in Erasme hospital, Brussels. PTLD was defined in conformity with WHO criteria. We analyzed baseline information, EBV history, PTLD characteristics, treatment and outcome.

Results
We identified 28 cases of PTLD. Two cases had no information concerning EBV status, one lacked data and was not considered. Average time from transplant at diagnosis was 6,22 years with 7 cases that had occured during the first year post-transplant (25,9%). Prior to development of PTLD, in those with information concerning EBV sero-status (25 cases), 6 had presented a seroconversion (24%) and 13 an EBV reactivation (52%). According to WHO criteria classification, 23 were monomorphic PTLD (85,2%), 3 were polymorphic PTLD (11,1%,) and 1 was an early lesions PTLD (3,7%). At diagnostis, the majority were a stage IV (63%); 22,2% a stage IE; 7,4% a stage I; 3,7% a stage II; 3,7% a stage III. Concerning treatment, 77,8% benefited from a reduction of immunosuppression; 77,8% were given rituximab (alone (37%) or combined with chemotherapy (40,8%)). After treatment, 14 achieved complete remission (CR), 3 had a partial response (PR), 3 had a progressive disease, 5 died soon after diagnosis, 2 were lost to follow-up. Regarding patients with CR, 5 relapsed (2 attained CR with second line treatment, 3 died). As to patients with PR, 1 died, 2 obtained CR after second line treatment.

Conclusion
Our series confirms a trend towards a late onset of PTLD (< 26 %

during the first year). 76% are concomitant with a history of EBV sero-conversion or reactivation. A Majority of the patients were already in advanced stages at diagnosis (monomorphic and stage IV).

P2.22 Monocytosis is associated with TET2 mutations in PTCL-NOS and AITL

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T lymphoma is a rare malignancy among all large cells lymphoma and an important therapeuticchallenge. Absence of cell lines makesthetranslationalapproachdifficult,andclinicalidentification of subgroups of patients and subsequent prognosis factors to individualize therapies seems essential. Two recent studies have shown a prognostic impact of a high monocyte count or of the presence of a TET2 mutation.First, in Bari et al. (Leukemia Research 37 (2013) 619-623), a monocyte count greater than 800/ml was correlated with a significant negative impact on overall survival in PTCL. Secondly, in Lemonnier et al. (Blood 120 (2012) 1466-1469), a subgroup of nombre of AITL and PTCL-NOS patients were carrying a TET2 mutation, and associated with a shorter PFS, compared to the non mutated patients. Here, we screened TET2 mutations for 6 patients with monocytosis, and 3 without within a group of 142 PTCL studied in hopital Henri Mondor.
151 patients were studied in one center between 2006 and 2010; median age was 57 years (20-81) and 67% were male. The median follow-up was 24 months. Patients of all histological subtypes were studied (PTCL NOS n=49, AITL n=41, ALCL n=33 (ALK+ n= 4), extranodal NK/T lymphomas n= 8, EATL n=3, HSTL n=3, SPTL n=2). At diagnosis, we found presence of monocytosis (> 800/mm3) among all T-lymphoma subtypes in 31% (n=48) of patients. 6 patients with monocytosis (PTCL-NOS=3, AITL=3) were screened for TET2 mutations. All were carrying a mutation: 3 frameshift, 1 frameshift + missense, 1 missense + frameshift + non-sense and 1 non-sense. 3 patients without monocytosis were screened as a control, but none of them were carrying a TET2 mutation.
We are currently screening more patients, but it seems on this small sample that a subgroup of patients with hypermonocytosis and TET2 mutations could be identified. PTCL could therefore, in their form with hypermonocytosis, share a common mechanism to myeloid disease, thus making the use of new epigenetic treatments as 5AZA used in MDS an evidence.

P2.23 Amaurosis as the inaugural sign of cerebral diffuse large B cell lymphoma in a HIV-1 patient

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Thrombotic complications are common in lymphoma. Chronic HIV infection is a major prothrombotic factor. However, acute visual loss as aninaugural sign of non-Hodgkin's lymphoma is a very rare phenomenon.
A 59-year-old patient with acquired immunodeficiency syndrome presented a sudden onset of unilateral amaurosis rapidly evolving in complete blindness. Physical examination was irrelevant. His treatment consisted in tenofovir, emtricitabine, rilpivirine. Blood tests identified a CD4 level at 530/µl and a viral load < 40 copies/ml. There was no other thrombotic factor. Carotid angiography revealed a unilateral central artery thrombosis of the retina. Aspirin and statin were prescribed without improvement. Two months later, he developed progressive headache. MRI revealed a unique fronto-temporal mass suspected to be malignant. Stereotaxic biopsy confirmed the diagnosis of diffuse large B cell lymphoma (DLBCL). Immuno-chemotherapy with R-COPADEM was initiated, in addition to intrathecal administration of methotrexate. Two weeks later, he complained again of headaches. CT scan identified a large pneumocephalus. MRI detected a dura mater gap that required a surgical closure. The patient completely recovered, but didn't show any visual improvement whereashe achieved a complete remission after 4 courses of treatment.
In conclusion, loss of vision is a rare presenting sign in DLBCL. Differential diagnosis is crucial as early diagnosis and effective treatment are important for survival as well as for visual restoration.

Posters myeloprofliferative disorders P3.01-P3.08

P3.01 SHP2 is required for hematopoietic cell transformation by FIP1L1-PDGFRA

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Activated forms of the platelet derived growth factor receptor alpha (PDGFRA) have been described in various cancers, including the FIP1L1-PDGFRA in patients with myeloproliferatitve diseases associated with hypereosinophilia and the $PDGFRA^{D842V}$ mutant in gastrointestinal stromal tumors.
To gain a better insight into the signal transduction mechanisms of PDGFRA oncogenes, we mutated each of the twelve potentially phosphorylated tyrosine residues of FIP1L1-PDGFRA. Mutation of tyrosine 720 in FIP1L1-PDGFRA or $PDGFRA^{D842V}$ inhibited cell growth and blocked MAP kinase (ERK1-2) signaling in Ba/F3 cells. This mutation also decreased myeloproliferation in transplanted mice and the proliferation of human $CD34^+$ hematopoietic progenitors transduced with FIP1L1-PDGFRA. Since the non-receptor protein tyrosine phosphatase SHP2 is known to bind to tyrosine 720 in wild-type PDGFRA, we evaluated its role downstream of FIP1L1-PDGFRA and $PDGFRA^{D842V}$. We found that SHP2 knock-down decreased proliferation of transformed Ba/F3 cells and ERK signaling, but not STAT5 phosphorylation. As reported before, SHP2 was not essential for cell proliferation and ERK phosphorylation induced by the wild-type PDGF receptor in response to ligand stimulation, suggesting a shift in the function of SHP2 downstream of oncogenic receptors.
In conclusion, our results indicate that SHP2 is required for cell transformation by mutant PDGF receptors and is a potential target for neoplasms carrying PDGFRA mutations that are resistant to tyrosine kinase inhibitors.

P3.02 Bone Marrow Stromal Cell-derived Exosomes Facilitate Multiple Myeloma Cell Survival Through Inhibition of the JNK Pathway

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Interplay between bone marrow stromal cells (BMSCs) and multiple myeloma (MM) cells plays a crucial role in MM pathogenesis by exchanging growth factors, cytokines, and other functional components. Exosomes are 30-100nm diameter membranous vesicles and mediate local cell-cell communication by transferring mRNAs, miRNAs, and proteins. Although the promotion of MM survival induced by BMSCs has been studied, the role of BMSC-derived exosomes in this action remains unclear. Here, we investigated the effect and mechanisms of murine BMSC-derived exosomes on the proliferation and survival of MM cells using the murine 5T33MM model.

Exosomes were isolated from conditioned medium after culture of primary BMSCs obtained from naïve C57BL/KaLwRij mice or 5T33MM diseased mice. The size of exosomes derived from naïve BMSCs, 5T33 BMSCs or 5T33MMvt cells were confirmed using a NanoSight LM10. Several exosomal markers such as CD63, Flotillin-1, heat shock protein 90 (HSP90), and HSP70 were detected. Both naïve and 5T33 BMSC-derived exosomes could fuse with 5T33MMvt cells. Several cytokines were found to be present in BMSC- and MMvt cell-derived exosomes. Both naïve and 5T33 BMSC-derived exosomes increased 5T33MMvt and MMvv cell viability and BrdU uptake. Significantly reduced apoptosis of 5T33 MMvt and MMvv cells were observed after exosomes' treatment. Bcl-2 was increased and activated (cleaved) caspase-3 was reduced after co-culture with BMSC-derived exosomes. Reduced phosphorylation of p53, p38MAPK and JNK were detected after naïve BMSC-derived exosomes treatment, whereas 5T33 BMSC-derived exosomes didn't change the activation of p53 and p38MAPK. 5T33 BMSC-derived exosomes further decreased the activation of JNK, Bim expression and phosphorylated Bim compared to naïve BMSC-derived exosomes. As Bim is a pro-apoptosis protein and mainly regulated by the JNK pathway, promotion of MM cell survival likely results from the inhibition of the JNK pathway by BMSC-derived exosomes. In summary, our results demonstrate a positive role for BMSC-derived exosomes in induction of MM cell proliferation and survival. BMSC-derived exosomes could inhibit the JNK pathway, thereby reducing caspase-3 activation and protecting MM cells from apoptosis.

P3.03 High-sensitivity multiparameter flow cytometric analysis for evaluation of bone marrow involvement in systemic mastocytosis

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Introduction

Systemic mastocytosis (SM) is characterized by neoplastic mast cells (MC) in one or more extracutaneous organs. Diagnosis is based on World Health Organisation (WHO) criteria including one major criterion (i.e. multifocal, dense infiltrates of ≥15 MC in bone marrow (BM) and/or extracutaneous organs) and four minor criteria: 1) >25% spindle shaped, immature or atypical MC in a biopsy or BM smear; 2) serum tryptase > 20 ng/ml; 3) p.D816V *KIT* mutation; and 4) expression of CD2 and/or CD25 on MC. One major and one minor criterion or three minor criteria are diagnostic for SM. We evaluated a highly sensitive flow cytometric assay on BM aspirates for its diagnostic utility in SM.

Methods

A protocol for immunophenotypic analysis of MC was developed.

Table 1: Characteristics of the patients with and without CD25 positive MC *(the gray shaded cells fulfill a WHO criterion)*

Patient	Age	Sex	Clinical Symptoms	BB	BM	Tryptase (ng/ml)	p.D816V/KIT mutation (%)	CD25 (%)	Final diagnosis
1	50	M	anafylaxis	nl	nl	28	0,002	0,020	ISM
2	41	F	UP + anafylaxis	SM	nl	20	0,010	0,005	ISM
3	53	M	anafylaxis	SM	SM	>200	0,020	0,090	ISM
4	40	F	UP	SM	SM	22	0,210	0,033	ISM
5	56	M	UP + anafylaxis	SM	SM	29	0,170	0,024	ISM
6	63	M	UP + osteolytic bone lesion	SM	nl	34	0,420	0,038	ASM
7	4	M	fever + fatigue + night sweating	nr	SM + AML	107	0,000	1,122	SM-AHNMD
8	27	M	anafylaxis	nl	nl	14	0,007	0,020	MMAS
9	38	F	urticaria + gastro-intestinal	nl	nl	23	0,000	0,002	MMAS
10	36	F	periodic fever + dermatosis	nl	nl	5	nd	0,000	no final diagnosis
11	52	F	facial erythema + food allergies	nl	nl	6	0,000	0,000	lactose intolerance + rosacea
12	2	M	urticaria + systemic inflammation	nl	nl	nd	0,000	0,000	auto-inflammatory syndrome
13	51	F	pruritus + rash	nl	nl	3	0,000	0,000	no final diagnosis
14	50	M	anafylaxis	nl	nl	40	0,000	0,000	MCAS
15	46	M	anafylaxis	nl	nl	5	0,000	0,000	no final diagnosis
16	57	F	elevated tryptase + non-specific symptoms	nl	nl	16	0,000	0,000	*H. pylori* gastric infection
17	28	M	gastro-intestinal	nl	nl	22	0,000	0,000	intestinal worm (oxyures)
18	65	F	allergies	nl	nl	14	0,000	0,000	no final diagnosis
19	55	M	urticaria + angio-edema	nl	nl	22	0,000	0,000	MCAS
20	56	F	anafylaxis	nl	nl	17	0,000	0,000	no final diagnosis
21	45	F	anafylaxis	nl	nl	14	0,000	0,000	no final diagnosis

BB: histopathology of bone biopsy; BM: cytology of bone marrow smear; SM: systemic mastocytosis; AML: acute myeloid leukemia; ISM: indolent systemic mastocytosis; ASM: acute systemic mastocytosis; SM-AHNMD: systemic mastocytosis with associated acute hematological non-mast cell disease; MMAS: monoclonal mast cell activation syndrome; MCAS: mastcell activation syndrome; UP: urticaria pigmentosa
nr: not representative; nl: normal; nd: not determined

P3.03 *Table 1*

BM was stained with CD34, CD117, CD45 and CD25 antibodies. Samples were analyzed on a FACSCanto II flow cytometer collecting ≥ 1 x 10^6 events. MC were identified by their high expression of CD117, intermediate CD45 expression and variable light-scattering characteristics. Co-expression of CD25 identified aberrant MC. Twenty-one patients with suspected diagnosis of SM were evaluated. Results were correlated with serum tryptase levels, BM cytology, histopathology of bone biopsies and *cKIT* p.D816V mutation status.

Results

Seven out of 21 patients met the WHO criteria for SM (all had 1 major and ≥2 minor criteria). In these seven patients, MC co-expressed CD25 (100% sensitivity) with a range of 0,002% - 1,122% (median 0,024%). Two patients not meeting the WHO criteria (only 2 minor criteria), showed clinical symptoms of MC degranulation and immunophenotypic abnormal MC (one had also the p.D816V mutation, the other had tryptase >20 ng/ml). Both cases might be considered as having the monoclonal mast cell activation syndrome (MMAS). The 12 patients without CD25 expression on the MC had no evidence of SM.

Conclusion

Highly sensitive multiparameter flow cytometry is a useful tool in the diagnosis of SM and might be helpful in diagnosing MMAS if <3 minor SM criteria are fulfilled.

P3.04 Screening JAK2 V617F-negative Essential Thrombocytosis Patients for Mutations in SESN2, DNAJC17, ST13, TOP1MT, and NTRK1

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Background

In a recent study by Hou et al., single cells derived from an ET *JAK2*V617F-negative ET patient were sequenced using a method based on exome sequencing. Eight genes were identified as possible candidate drivers.

Aims

To establish the recurrence rate in *JAK2* V617F-negative and *MPL* W515K/L-Negative ET of potential candidate driver mutations, as identified by Hou et al.

Methods and Results

We studied unfractionated blood or bone marrow samples from a

series of 64 cases of *JAK2* V617F-negative and *MPL* W515K/L-negative ET. In this series, we used Sanger sequencing to detect the following mutations: SESN2 P87S, *TOP1MT* S479L, *ST13* Q349*, and *DNAJC17* A292P, as they exhibited the highest scores in the study of Hou et al. In addition, we included *NTRK1* N323S, a mutant tyrosine kinase. None of the mutations reported by Hou et al. was detected in our patients. However, we identified a novel acquired heterozygous mutation in *TOP1MT* (c.1400A>G, p.N467S). TOP1MT is a mitochondrial topoisomerase encoded by the genomic DNA. This mutation might affect the interaction of TOP1MT with the DNA molecule as suggested by *in silico* analysis, due to the gain of a α helix and the loss of a β strand which are in close proximity to the bound DNA molecule. We screened exon 11 of *TOP1MT* gene in 38 additional *JAK2* V617F-negative *MPL* W515K/L-negative ET cases, but did not find any additional cases.

Conclusions
In this series of 102 ET cases, only one case was identified with a mutation of *TOP1MT*. Mutations of *SESN2, ST13*, *DNAJC17*, or *NTRK1*, as identified by Hou et al., could not be identified in a series of 64 cases. The functional role of *TOP1MT* in the patho-genesis of ET remains to be established. The absence of the mutations, as proposed by Hou et al., in our cohort raises questions about their role as potential driver mutations in *JAK2* V617F-negative ET.

P3.05 Efficacy of azacitidine in Belgian patients: results of a real-life non-interventional, post-marketing survey

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Myelodysplastic syndromes (MDS) are haematopoietic stem-cell disorders affecting older adults. Approximately 50% of MDS patients progress to acute myeloid leukaemia (AML). Azacitidine (Vidaza®) is approved to treat high-risk MDS, AML (with 20-30% blasts) and chronic myelomonocytic leukaemia (CMML). Here we report the efficacy of azacitidine in MDS, CMML or AML patients treated in a real-life setting. Safety, a primary endpoint, will be described in detail elsewhere (manuscript in preparation).
This non-interventional, post-marketing survey included 49 patients recruited in 14 Belgian haematology centres between 2010 and 2012. Baseline patient and disease characteristics were collected. At the end of 1-year observation period (1YOP) or at treatment discontinuation (TD), whichever occurred earlier, we evaluated treatment response (complete response [CR], partial response [PR], haematological improvement [HI], stable disease [SD], treatment failure [TF]), transfusion-independence (TI; the absence of transfusion of red blood cells or platelets during at least 8 weeks), and overall survival (OS).
77.5% of patients were ≥65 years old (mean age, 72.5 years); 69.4% were male. The ECOG performance status was 0 for 24.5%, 1 for 44.9% and 2 for 18.4% of patients. 69.4% had MDS, 26.5% had primary or secondary AML and 4.1% had CMML. During 1YOP, patients received a median of 7 treatment cycles. Among MDS and CMML patients (n=29), 41.4% had CR, PR or HI, 41.4% had SD and 17.2% had TF. Among AML patients (n=9), 44.4% had CR or PR, 33.3% had SD and 22.2% had TF. TI was observed in 14/32 (43.8%) patients. 71.4% of patients discontinued treatment before the 1YOP; median time-to-treatment cessation due to non-response, TF, adverse event or death was 308 days. Median (95% confidence interval) OS was 490 days (326-555); 1-year OS estimate was 0.571 (0.422-0.696). The azacitidine safety profile was consistent with previous findings.
These results are in line with previously reported real-life data, suggesting that azacitidine prolongs the survival of MDS, AML or CMML patients.
Vidaza® is a registered trademark of Celgene Corporation

P3.06 Les toxidermies à l'imatinib. Etude retrospective a propos de 28 cas

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L'imatinib est un inhibiteur de tyrosine kinase de 1ere génération utilisé actuellement comme traitement de 1ere ligne dans la Leucémie Myéloïde Chronique (LMC). Des effets secondaires hématologiques et extra-hématologiques (digestifs, hépatobiliaires, généraux et dermatologiques) sont décrits avec l'imatinib. Les réactions cutanées à l'imatinib sont fréquentes et surviennent chez 11 à 67% des patients selon les séries.
Patients et méthodes : Nous avons mené une étude rétrospective sur une période de 24 mois entre aout 2010 et aout 2012 intéressant les patients atteints de LMC suivis au service d'hématologie à Casablanca. L'objectif de notre étude est de décrire les différentes atteintes dermatologiques secondaires au traitement par Imatinib.
Résultats : 80 cas de LMC ont été traités par Imatinib durant cette période. La moyenne d'âge de la population étudiée est de 44 ans avec des extrêmes allant de 22 mois à 80 ans. Le sexe ratio H/F représente 0.78. 66% des patients sont en phase chronique, 5% sont en accélération et 11% en transformation aigue au moment du diagnostic. 28 cas (35%) ont manifesté une réaction cutanée à l'imatinib. L'2'dème palpébral ou bouffissure du visage sont retrouvés chez 14 cas, l'hypochromie du visage dans 8 cas, une éruption maculo-papuleuse dans 3 cas, un prurit dans 2 cas et une lésion ulcérée de la cheville diagnostiquée comme sarcome de kaposi dans un cas. Le traitement de ces toxidermies a conduit à un traitement symptomatique chez 24 patients, un arrêt temporaire de l'imatinib a été note dans deux cas, un arrêt définitif dans un cas avec utilisation des ITK2 de 2 eme génération puis de l'interféron et la greffe cutanée a été sollicitée pour traiter le sarcome de kaposi qui a bien évolué sous imatinib.
Les toxidermies à l'imatinib sont des réactions secondaires fréquentes qui doivent être recherchées avec soin chez tout patient atteint de LMC sous traitement.

P3.07 Feasibility of analyzing BCR-ABL1 transcripts on bone marrow samples using the GeneXpert system

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Introduction
For monitoring of *BCR-ABL1* transcripts in CML patients the ELN 2013 guidelines recommend to perform quantitative *BCR-ABL1* analysis on peripheral blood (PB) samples. In our laboratory, these samples are run on a GeneXpert system using V1 cartridges (Cepheid). However, laboratories might also receive bone marrow (BM) samples for quantification of *BCR-ABL1*. Although the manufacturer does not support BM analysis on GeneXpert, the aim of this study was to evaluate the feasibility of *BCR-ABL1* quantification in BM using the GeneXpert.

Materials and methods
Thirty-seven CML follow-up BM samples were tested both with GeneXpert and TaqMan-RQ-PCR aligned to IS (international scale). GeneXpert analyses were performed according manufacturers recommendations, but only 20 µL of sample was used. Results were expressed as *BCR-ABL1* %IS. To compare results we used a categorical interpretation with following logarithmic intervals: 10-1%, 1-0.1%, 0.1-0.01%, 0.01-0.001%, <0.001% and "not detected".

Results
Nineteen out of 37 samples (51.4%) had the same categorical interpretation and 14 samples (37.8%) had one categorical difference. For 10 out of these 14 samples, there was MMR (major molecular response) by both methods, 3/14 showed no MMR by both methods and in 1/14 there was a discordance; no MMR with TaqMan and MMR with GeneXpert. Four samples (10.8%) showed a difference of ≥ two categories. Out of these 4 samples, 2 had MR4 (molecular response of ≥4 log reduction) and 1 had MMR with TaqMan, while no *BCR-ABL1* with GeneXpert. The fourth sample had no MMR with TaqMan (0.221%IS) while GeneXpert showed no detectable *BCR-ABL1*; this due to a suboptimal GeneXpert PCR, as indicated by low copies of GUS. Looking at the double positive samples, 24/25 (96%) showed comparable stratification of molecular response (no MMR, MMR).

Conclusions
Although the current GeneXpert assay might be slightly less sensitive than TaqMan PCR, in case of positivity stratification of molecular responses are comparable. Therefore, quantification of *BCR-ABL1* in BM on GeneXpert is feasible and might be an alternative for time consuming in house methods.

P3.08 Successful renal transplantation after autologous hematopoietic stem cell transplantation in anuric multiple myeloma

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Multiple myeloma (MM) is a treatable, although incurable disease. Kidney impairment is common in MM, since 20 to 30% disclose renal impairment at diagnosis and up to 2-5% will need long-term dialysis.
We report on the case of a 67-year-old woman diagnosed with Salmon-Durie stage IIIB, ISS III IgG kappa MM, normal karyotype, who was initially referred for renal failure with anuria associated with gastroenteritis. The patient was under non-steroidal anti-inflammatory drugs for laterothoracic bony pain. Her physical examination was irrelevant except for systolic high blood pressure (160/70 mmHg). Blood tests showed mild anemia (hemoglobin at 8 g/dl), renal failure (serum creatinine at 16 mg/dl) and identified an IgG kappa M-protein. Cast nephropathy was confirmed on kidney biopsy. Hemodialysis was required thrice a week in addition to chemotherapy with bortezomib-dexamethasone (VD). Very good partial response was achieved after 4 cycles, without renal recovery. Intensification with intermediate dose melphalan (140 mg/m²) followed by autologous hematopoietic stem cell transplantation (ASCT) was then performed. The procedure was complicated by a grade 4 mucositis and pneumonia with ARDS favored by bone marrow regeneration. Complete response was achieved after consolidation with 2 additional cycles of VD, however without renal recovery. Because of her excellent performance status, the absence of co-morbidities, and after a complete work-up, she was considered for renal transplantation. One-year post-ASCT, as she remained in CR, she received a kidney transplant from a deceased donor. Seven months after renal transplantation, she enjoys a normal life and remains in CR.
Although prognosis of MM has improved during recent years with the advent of ASCT and novel drugs, patients with renal failure are often excluded from aggressive or high-dose therapy protocols because of an expected higher toxicity. We report on the case of a MM patient who failed to recover from cast nephropathy despite the use of new agents, underwent an ASCT without major complications, and was successfully transplanted with a cadaveric kidney.

Posters immunology, stem cell biology and transplantation
P4.01-P4.19

P4.01 Zinc finger nuclease (ZFN)-mediated FANCA disruption reveals severe growth disadvantage of FANCA knockout human embryonic stem cells (hESC)

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Fanconi anemia (FA) is characterized by progressive bone marrow failure (BMF), leading to acute myeloid leukemia (AML) in 30% of cases. To investigate the mechanism underlying hematopoietic defects seen in FA, we aimed to create a human pluripotent stem cell model by knocking out *FANCA* in hESC via ZFN-mediated homologous recombination (HR).
After targeting *FANCA* by inserting a Puromycin (Puro) selection cassette, we found one clone where Southern blot (SB) suggested a higher abundance of transgenic (TG) alleles than wildtype (WT). Given that hESC are split as clumps, we hypothesized that this clone might represent a mixture of mono-allelically (+/-) and bi-allelically (-/-) targeted cells. Disappearance of the band corresponding to the WT allele upon reselection with a higher dose of Puro confirmed this hypothesis. However, when the same clone was assessed by SB a few passages after Puro selection, the WT allele was again detected, suggesting a selective growth advantage of +/- cells, which can escape or withstand selection pressure, over -/- cells.

Next, we performed HR with a Hygromycin (Hygro) cassette targeting the WT allele of previously Puro targeted clone. Hygro^R clones in which bi-allelic targeting was confirmed based on the absence of the WT allele underwent growth arrest and could not be maintained in culture. Some feeder-free subcultures however could be maintained. In these cultures, the WT allele could again be amplified and remained present despite extensive reselection. Single-cell dissociation with the aim of establishing clonal cultures rapidly triggered growth arrest. The presence of WT *FANCA* was further substantiated by Western blot and immunofluorescence.
Together these data point towards a severe growth disadvantage of *FANCA* knockout hESC and suggest that growth disadvantage of FANCA -/- allows for the persistence of cells still having WT *FANCA* despite extensive selection. Although this coexistence seems to permit the *FANCA* knockout cells to be maintained in culture longer, the heterogeneous nature of the culture prevents accurate characterization and establishment of a human pluripotent FA disease model.

P4.02 The effect of multipotent adult progenitor cells on bone marrow failure in myelodysplastic syndromes

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Introduction
Primary myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell (HSC) disorders characterized by ineffective hematopoiesis and peripheral cytopenias. Intrinsic defects in the HSC as well as extrinsic defects in the bone marrow (BM) niche all contribute to the MDS pathogenesis. In some patients, immunomodulatory drugs showed a significant improvement in cytopenias. Multipotent Adult Progenitor Cells (MAPC) are non-hematopoietic stromal stem cells derived from BM with potent immunomodulatory effects towards T cells.

Aim
Test the effect of MAPC as a cell-based therapy for low-risk MDS patients.

Materials and Methods
Two MDS mouse models were generated: a first model by overexpressing the oncogene Evi-1 in lin^- cells of murine BM that were subsequently transplanted into lethally irradiated C57Bl/6 mice. A second model by intercrossing floxed Dicer with Osterix-Cre mice to create Osx-$Cre^+Dicer^{lox/lox}$ (OCD) mice, in which Dicer is selectively deleted in BM osteoprogenitors. Furthermore, BM samples from MDS patients were used in hematopoietic cultures with or without human MAPC.

Results
In Evi-1 transplanted mice, pancytopenia developed 10-15 months after BM transplantation and Evi-1 was highly expressed in blood and BM. The number of hematopoietic colonies and frequency of primitive progenitors was decreased in these mice. The OCD model showed lower blood counts, a decreased number of mixed colonies and lower frequency of hematopoietic progenitors as compared to control mice. Morphological analysis revealed dysplastic megakaryocytes in BM and increased polychromatophilic RBCs in blood of diseased mice.
A short-term culture with total BM cells from MDS patients showed an increase in CFU-GM colonies when MAPC were added to the culture. Similarly, when $CD34^+$ cells from these patients were plated on feeders in a long-term culture, we could observe a higher frequency of primitive hematopoietic progenitors in the condition with MAPC.

Conclusion
We were able to establish two mouse models of MDS (the Evi-1 and OCD model) and could show a positive effect of human MAPC on the *in vitro* colony forming capacity of hematopoietic cells derived from MDS patients.

P4.03 Identifying novel regulators hematopoietic stem cell expansion ex vivo

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Maintenance of hematopoietic stem cells (HSC) self-renewal ex vivo remains a major challenge in order to exploit sources with limited numbers of HSCs, such as umbilical cord blood, clinically.
Self-renewal is a unique attribute of stem cells to maintain the pool of stem cells own identity during cell division without losing its ability to remain multi(pluri)potent (asymmetrical divisions), or lead to expansion of the stem cell pool (symmetrical divisions). Both intrinsic and extrinsic factors can influence the self-renewal capability of HSC. Here, we performed genome-wide transciptome analysis of fetal and adult bone marrow mouse derived HSC and the fetal liver niche (at e12.5-16.5) to identify novel factors involved in symmetrical divisions and hence expansion of HSC *in vitro* using RNA-Seq. Transcriptional regulators were shortlisted after comparing HSC isolated from e14 fetal liver and adult bone marrow. The role of these intrinsic regulators in hematopoiesis was screened by morpholino knockdown studies, and subsequent RT-PCR and in situ hybridization using Gata-1(dsred)/Flk(GFP) transgenic Zebrafish lines. Cell extrinsic factors identified by RNAseq from laser-captured microdissection cells that surround Lin-/CD11b+/Sca1+ cells (expressed in e14 fetal liver but not either in e12.5 or e16.5 fetal liver) for which receptors were found on HSC were also shortlisted. The extrinsic regulators were screened for their role in maintenance of HSC self-renewal using an ex vivo culture system wherein the identified secreted molecules were added. The ongoing functional validation of transcriptional regulators in HSC using fetal liver HSC niche extrinsic factors will provide novel insights in mechanisms that support symmetrical selfrenewal of HSCs, which may be of great interest for clinical HSC transplantation, gene therapy, or creation of mature blood cells from stem cells.

P4.04 Allogeneic stem cell transplantation for patients with myelofibrosis: a single center experience

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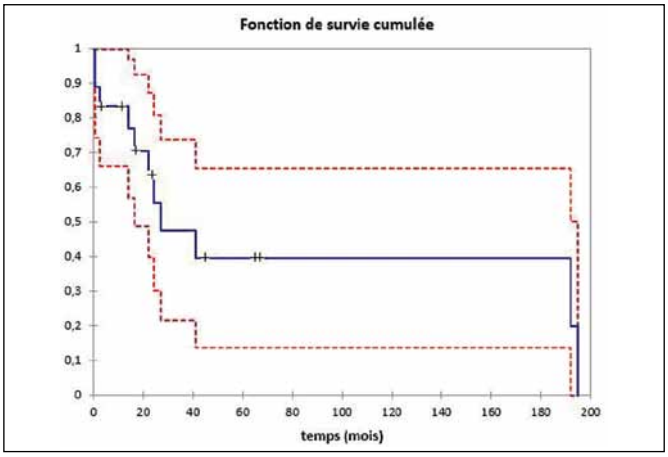
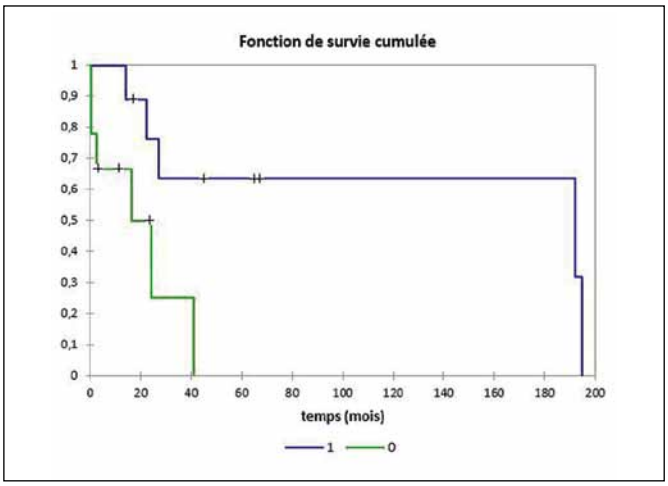
Allogeneic stem cell transplantation (SCT) is the only curative treatment for myelofibrosis (MF). We retrospectively evaluated patients who underwent SCT for MF in our institution between 1985 and February 2013.
We analyzed 18 patients with a M/F ratio of 13/5 and a median age at SCT of 53.8 years old (range 41,7 - 64,8). Diagnostic was primary MF in 13, and secondary/transformed MF in 5. Risk stratification according to DIPSS+ was: intermediate-1 5,5%; intermediate-2 38,8%; and high 44,4%. JAK V617F mutation was present in 4/11 evaluable patients. Donors were: matched sibling, 50%; HLA-matched unrelated, 50%. Intensity of the conditioning regimen was myeloablative (MA) in 8 and reduced (RIC) in 10.
Overall, 16/18 patients engrafted. Complete chimerism occurred in 12/15. Acute and chronic graft-versus-host-disease (aGVHD and cGVHD) were seen in 6/18 and 9/18 patients, respectively. Median follow-up of the patients was 22,9 months. Median progression-free survival (PFS) and overall survival (OS) were 24 months and 27 months, respectively (Figure 1A). Eleven patients died, 8 from non-relapse mortality (NRM). Outcomes were not different according to year of SCT, age, gender, donor type, SCT type, and presence of aGVHD. There was a slight trend towards better PFS (log-rank 3.7, *P* 0.056) in patients with a shorter interval to SCT. OS was improved (Log-rank 4.852, *P* 0.028) in patients with intermediate DIPSS+ score. Both PFS (Log-rank 9.130, *P* 0.003) and OS (Log-rank 5.196, *P* 0.023) were improved in patients with cGVHD (Figure 1B).
Our results show that allogeneic SCT for MF induces long-term survival in about 40% of the patients. Still, NRM remains very high. Multivariate analysis was not performed due to the small patients number but univariate analysis indicate a potential



P4.04 *Figure 1.* OS of 18 MF patients transplanted with allogeneic donor at Saint-Luc



P4.04 *Figure 2.* OS according to occurrence of cGVH (blue line) or absence of cGVH (green line)

better outcome if cGVHD occurs. Survival may also be improved in patients with lower DIPSS+ score and shorter interval to SCT. In conclusion, allogeneic SCT brings the potential for cure, probably via graft-versus-MF effect, but efforts are warranted to lower NRM and better select patients.

P4.05 High incidence of second malignancies in patients given allogeneic hematopoietic stem cell transplantation following reduced-intensity (RIC) or nonmyeloablative conditioning

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Purpose

Allogeneic hematopoietic stem cell transplantation (allo-SCT) following nonmyeloablative or reduced-intensity conditioning regimens has been increasingly used as treatment for patients with hematological malignancies who are too old or too sick to tolerate high-dose myeloablative allo-SCT.

Patients and Methods

Here we review the data of 287 consecutive patients given allogeneic bone marrow or peripheral blood stem cell (PBSC) transplantation after nonmyeloablative or reduced-intensity (RIC) conditioning at our center from 1999 to 2012, and specifically focused on factors affecting long-term OS on the one hand and on the occurrence of second malignancies on the other hand.

Results

Twenty-one patients (7.3%) experienced graft rejection, while the remaining 266 patients had sustained donor engraftment. Acute GVHD of grade II, III and IV were seen in 69 (24%), 15 (5%) and 19 (7%) of patients, respectively. Moderate or severe chronic GVHD occurred in 44 (15%) and 65 (23%) patients, respectively. One-, two-, five-, and ten-year OS were 65%, 55%, 40% and 29 %, respectively. Factors associated with improved OS included high Karnofsky score (P<0.0001), younger patient age at transplantation (P=0.036), low disease risk (P=0.029), being given graft from HLA-identical siblings (P=0.031), and being transplanted after 2007 (P=0.005). The 2-, 5-, and 10-year probabilities of having a second malignancy were 7%, 15% and 35%, respectively, and second cancers were one of the leading causes of late mortality after transplantation.

Conclusions

These data demonstrate that nonmyeloablative / RIC allo-SCT is feasible in patients up to 70-75 years of age, but that occurrence of second malignancies is a serious concern in long-term survivors.

P4.06 Identification of Integrin-av as an important marker of primitive hematopoietic stem cells in mouse and human

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Integrins play important roles in maintenance of hematopoietic stem and progenitor cells (HSPCs) in addition to aiding in the homing of transplanted HSPCs into the BM niche. Their interactions with extra cellular matrix components, other cell surface molecules expressed in the niche or secreted molecules have been shown to regulate HSPC function. Here, we describe the identification of Integrin-αv (Itgav) as an important marker to enrich primitive hematopoietic stem cells (HSCs) within the mouse BM derived Lin^- $c\text{-}kit^+Sca\text{-}1^+CD48^-CD150^+$ (SLAM-LSK) sub-population. The mouse (m)SLAM-LSK cells that expressed Itgav showed higher potential of long-term engraftment when transplanted in lethally irradiated mice in competitive repopulation assays compared with their Itgav- counterparts. Similar experiments with human umbilical cord blood derived cells showed that the sub-population of $Lin^-CD45^+CD34^+$ $CD38^-$ cells that expressed Itgav exhibited better long-term engraftment and higher frequency of long-term culture-initiating cells (LTC-ICs) that represent primitive HSPCs in vitro.

Itgav acts as a receptor for Periostin (Postn, also known as Osteoblast specific factor-2; OSF-2) expressed in the BM niche. In vitro experiments demonstrated that Postn inhibits cell cycle progression of mHSPCs via up-regulation of $p27^{Kip1}$ expression through the Akt pathway, which increased the proportion of primitive mHSCs following 5 days culture of mSLAM-LSK cells. Preliminary studies demonstrated that in *Postn*$^{-/-}$ mice, increased frequency of myeloid progenitor but decreased frequency of mSLAM/LSK cells was detected. Experiments with specific deletion of Itgav in hematopoietic system are ongoing to better understand the mechanisms involved.

Although HSC function and their proliferation have been related to integrin expression and their attachment to the niche, this is the first report of the direct regulation of proliferation of HSCs by outside-in integrin signaling.

P4.07 Clinical and Immunological Predictive Factors of Late Infection Burden After Alternative Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

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Background

Mismatched unrelated donor (MMUD) or umbilical cord blood (UCB) can be chosen as alternative stem cell sources for HSCT. However, they may be associated with prolonged immune deficiency and risks of infections.

Methods

Patients transplanted from UCB (n=30) or MMUD (n=36) at Saint-Louis Hospital (Paris) during 2005-2010 were evaluated. Immune cells phenotypes were prospectively assessed on fresh blood samples at 0, 3, 6 and 12months after transplant. Detailed analyses of late (>3months posttransplant) infections were also performed.

Results

A prolonged CD4+ T-cell deficiency was observed after both MMUD- and UCB-HSCT (median counts <0.5x10^9/L by 1year posttransplant). Compared with MMUD-HSCT, UCB-HSCT was characterized by higher numbers of NK-cells at 3 and 6 months and lower counts of T-cells (particularly CD8+ T-cells) up to 1 year post-transplant.

The 18-month cumulative incidences of late infections and of infection-related death were high in both groups: 72 versus 57% and 10 versus 14% after MMUD- and UCB-HSCT, respectively (P=NS). Rate of infection per 12 patient-month was 2 (1 bacterial, 0.9 viral and 0.3 fungal).

We further tried to identify early clinical and immunological prognostic factors (defined at baseline and at 3 months post-HSCT) of late infections. The graft source did not impact infection risks. The 3-month cumulative corticosteroid dose and the absolute CD4+ T-cells count at 3 month were the sole predictors. Higher risks were observed with increased corticosteroid exposure while higher CD4+ T-cell was protective against later infections. Eventually, by designing a joint model of immune cell subsets' evolution over the first 12 months after HSCT and hazard of first late infection, we observed that the progressive increase in B-cells and in CD4+ memory T-cells counts was associated with reduction in infection risks.

Conclusion

Patients transplanted from alternative graft sources represent a population with high risk of late infections. The 3-month cumulative corticosteroid exposure is a risk factor for infections. Phenotypic analysis of immune cells at 3 months posttransplant may help to evaluate infection risks and to adjust prophylaxes.

P4.08 Disease risk index fails to predict survival after allogeneic peripheral blood stem cell transplantation (PBSCT) - Are multiple myeloma and myeloproliferative neoplasms misclassified?

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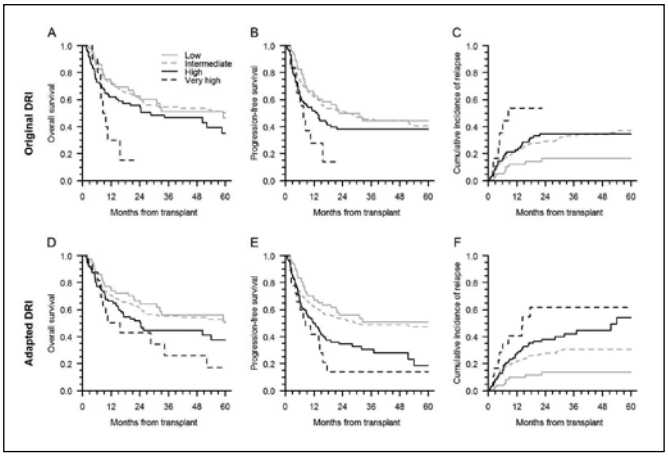
Background

Outcomes after PBSCT for hematologic malignancies may considerably vary according to the disease type and the remission status at the time of transplant. Recently, Armand et al. have proposed the *Disease Risk Index* (DRI), a new tool for risk-stratifying patients with respect of overall survival (OS) and progression-free survival (PFS) basing on primarily differences in relapse risks (*Blood 2012*).



P4.08 *Figure 1.* Outcomes according to DRI (A, B, C) and aDRI (D, E, F); OS (A, D), PFS (B, E) and cumulative incidence of relapse (C, F)

Methods

We retrospectively analyzed outcomes of 442 patients with hematologic malignancies who underwent PBSCT after myeloablative or reduced intensity conditioning at Saint-Louis Hospital (Paris) during 2000-2010 and we evaluated DRI as a predictor for OS and PFS. Diagnoses included acute myelogenous leukemia (122), non-Hodgkin lymphoma (62), myelodysplastic syndrome (60), multiple myeloma (MM) (57), acute lymphoblastic leukemia (40), myeloproliferative neoplasms (MPN) (37), Hodgkin disease (29), chronic myeloid leukemia (20) and chronic lymphocytic leukemia (15). Overall, 59 (13%), 279 (63%), 91 (21%) and 12 (3%) patients were classified as having a low, intermediate, high and very high DRI, respectively. Median follow-up time after transplant was 36 months (range 2-133).

Results

DRI did not fully stratify patients for OS and PFS as no significant difference was observed for patients with low, intermediate and high DRI, in both univariate (**Figure 1**) and multivariate analyses.

In the original publication, diseases that were underrepresented were randomly assigned into the intermediate disease type risk category. These included MPN and MM, that were relatively more represented in our cohort. When assessing both diseases, we observed that MPN and MM patients had respectively better and worse outcomes than patients assigned to the intermediate disease type risk category. Therefore, we constructed an "*Adapted* DRI (aDRI)" in which MPN and MM were respectively assigned to the low risk category and to the high risk category for disease type.

OS, PFS and CIf of relapse stratified by aDRI are shown in **Figure 1**. Compared with the original DRI, aDRI better stratified relapse and PFS but not OS (integrated discrimination improvement index: 0.060, 0.039, -0.002, respectively).

P4.09 A novel HLA-C null variant allele (C*05:XXN) generated by single nucleotide deletion and premature stop codon in exon 3

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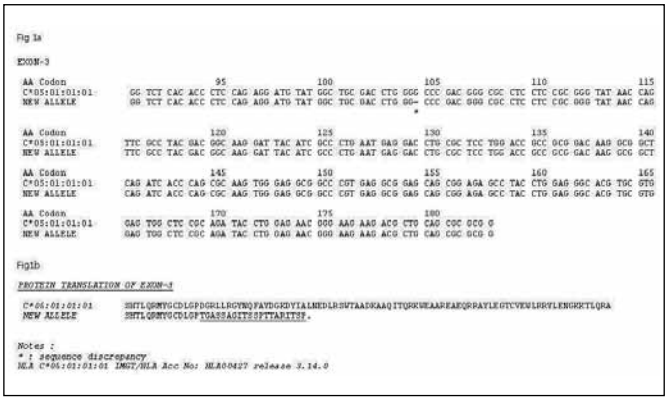

P4.09 *Figure 1*

We describe the detection of a variant HLA-C null allele during routine HLA typing of a Caucasian hematopoietic stem cell recipient. Low resolution HLA typing was performed by HLA SSO typing kits (Immucor©), showing HLA-C*05,07. Subsequent high resolution confirmatory typing by sequence based typing (SBT) of exons 1 to 7 using Gendx© SBT reagents revealed genotypic ambiguities C*05:05, C*07:01:01:01; C*05:01:01:01, C*07:18 and C*05:01:03, C*07:166. No group specific sequencing primers (GSSPs) were available to resolve these ambiguities. Subsequent allele specific sequencing of exons 1 to 4 using Protrans© for locus HLA-C showed C*05:01:01:01, C*07:01:01:01 with allelic ambiguities. Most of these ambiguities could be excluded by SBT Gendx except for C*07:18. C*07:18 differs from the C*07:01:01:01 allele by a unique nucleotide position within exon 6, showing a heterozygous position for which no allele specific primers were available. Further analysis using PCR-SSP confirmed the presence of the HLA C*07:18 allele with C*05:01. The new HLA C*05:XX allele results from a single nucleotide loss at position 381 in exon 3, codon 104 of the HLA-C gene, compared to its closest allele C*05:01:01:01 (fig 1a). This deletion causes a frame shift and premature stop codon(TGA) at codon 126 (fig 1b) and therefore assigned a HLA null allele. HLA null alleles are characterized by the lack of a serologically detectable product. These null alleles result from mutations in introns, exons or promoter sequences. Because serological HLA diagnostics are increasingly replaced by DNA-based typing methods considering only small regions of the genes, null alleles may be misdiagnosed as normally expressed variants. The failure to identify an HLA null allele as a non-expressed variant in the stem cell transplantation setting may result in an HLA mismatch that is highly likely to stimulate allogeneic T cells and to trigger GVHD. Therefore, patients carrying a HLA null allele, should be considered homozygous for the other, normally expressed allele in donorsearch. The finding of this new HLA-C*05:XX null allele highlights the importance of SBT as the ultimate technique, for identifying new HLA alleles.

P4.10 Imatinib improves survival of chronic Graft-versus-Host disease by inhibiting TGF-beta and PDGF-r pathways in mice

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Introduction

Graft-versus-host disease (GVHD) remains a major complication of allogeneic hematopoietic stem cell transplantation (HSCT). Approximately 15% of the patients develop the sclerodermatous cGvHD (scl-cGvHD) form of the disease characterized by multiple organ fibrosis and loss of skin elasticity. A few studies have suggested potential benefits of imatinib, a tyrosine kinase inhibitor, as a treatment of fibrosis in scl-cGVHD due to its ability to inhibit simultaneously the PDGF receptor and TGF-β pathways (via ABL inhibition), which are both involved in fibrosis.

Aim

This work investigates the impact of imatinib on fibrosis in the B10.D2 to BALB/cJ scl-cGvHD murine model. Lethally irradiated BALB/cJ recipient mice were injected with 10^7 bone marrow cells + 7.10^7 splenocytes from B10.D2 donor mice. Recipients were treated with imatinib 150 mg/kg/day (50 mg/kg in the morning followed by 100 mg/kg in the evening) by oral gavage or the same volume of sterile water. Mice health status was evaluated with a scoring system encompassing five criteria (weight loss, activity, fibrosis, hair loss and mice posture; 0-1-2 points/criteria). Mice were sacrificed at a score of 8/10 according to our local ethical committee.

Results

Mice given daily 150 mg/kg imatinib had a better survival than control mice (42 versus 33 days, p = 0,0357). cGvhD scores were suggestively lower in imatinib-treated than in control mice ($p \leq$ 0,15). Further, histological analyses evidenced reduction in the levels of both PDGF receptor (p = 0,033) and c-Abl (p = 0,185) phosphorylation in imatinib as compared to control mice. Finally, no significant differences were observed in the number or frequency of lymphocyte subsets in the 2 groups of mice.

Conclusion

Imatinib slightly decreased fibrosis and significantly improved survival in a severe scl-cGvHD murine model.

P4.11 Genetic correction of induced pluripotent stem cells from a Wiskott-Aldrich Syndrome patient normalizes the immune defects

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Introduction

Wiskott-Aldrich syndrome(WAS) is an X-linked primary immunodeficiency disease characterized by thrombocytopenia, recurrent infections and increased autoimmunity. This disease is caused by mutations in the WAS protein(WASp) gene which is exclusively expressed in hematopoietic cells and results in functional abnormalities in lymphoid cells and platelet production. Currently, these patients are treated with allogeneic stem cell transplantation or lentiviral hematopoietic stem cell gene therapy(1). We investigated restoration of T- and NK-cell functionality using the zinc-finger nuclease(ZFN) targeted gene-correction strategy.

Results

We generated induced pluripotent stem cells(iPSC) from fibroblasts of a WAS patient carrying an insertional mutation. Sub-sequently, a WAS-2A-eGFP transgene was targeted at the endogenous chromosomal location using ZFN-technology, thereby correcting the gene defect and creating a GFP reporter for WASp expression. Hematopoiesis from WAS iPSC and gene-corrected iPSC(cWAS) was studied. A human embryonic stem cell line WA01 was used as control.

Making use of spin embryoid bodies, hematopoietic differentiation was induced in cWAS iPSC. Weak GFP expression was first noted in CD34+CD43-KDR+ hemogenic endothelium and was pronounced in all CD43+ hematopoietic lineages including myeloid, monocytic,

lymphoid, erytroid and megakaryocytic lineages.
Hematopoietic precursors were cultured on OP9-DL1 to generate T and NK cells. NK cells were readily obtained from WAS, cWAS and WA01. On the other hand, only low numbers of TCR$\alpha\beta$ and TCR$\gamma\delta$ cells were obtained with WAS compared to WA01. T-cell generation was restored in cWAS. Likewise WAS generated NK cells were unable to generate interferon-γ or tumor necrosis factor-α upon stimulation with K562. Cytokine production was restored in cWAS generated NK cells.

Conclusion
Targeted endogenous integration of the WAS gene in WAS-iPSC results in restoration of the lymphoid defect observed in WAS-iPSC. Transplantation of gene-corrected iPSC-derived hematopoietic precursors may offer an alternative to lentiviral gene therapy which carries an inherent risk for selection of integrations near oncogenes and aberrant clonal expansion.

1) Aiuti A, et al. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome.Science.2013.

P4.12 A case of transplantation-associated thrombotic microangiopathy, with surprisingly suppressed ADAMTS-13 activity

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We present here the case of a 25-year old female with severe aplastic anaemia, relapsing after immunosuppressive therapy (ATG and cyclosporin). She underwent a haematopoietic stem cell transplantation (HSCT) with a matched unrelated donor after reduced intensity conditioning with busulphan and cyclophosphamide. Immunosuppression consisted of methotrexate and cyclosporin. The HSCT was complicated by the development of an overwhelming invasive pulmonary aspergillosis, with the need for invasive mechanical ventilation. On day 21 post-HSCT, she was diagnosed with a thrombotic microangiopathy (TMA), evidenced by the presence of schistocytes, thrombocytopenia, elevated lactate dehydrogenase (LDH) and bilirubin, complicated with acute renal insufficiency. At that time, the cyclosporin level was within therapeutic ranges. The diagnosis of transplantation-associated TMA was suspected and cyclosporin was replaced by mycofenolic acid. Surprisingly however, ADAMTS-13-activity was suppressed (< 0.55%), indicative of a true TTP (thrombotic thrombocytopenic purpura). Upon this diagnosis, the patient was treated with plasmapheresis in combination with corticosteroids. Upon this treatment, the acute renal insufficiency resolved and the diagnostic features of TTP disappeared. The patient recovered from the pulmonary complications. She was discharged at day 80 post-HSCT and is still alive and in good condition on day 228 post-HSCT.
Thrombotic microangiopathies can be subdivided in transplantation-associated TMA (TA-TMA) and non-transplantation-associated TMA (NTA-TMA). Within NTA-TMA, the classical idiopathic TTP is the most frequent, and is attributed to a deficient activity (<5%) of the von-willebrand-factor cleaving-protease (ADAMTS-13), due to the presence of autoantibodies. Therefore, plasma exchange is part of the standard treatment in NTA-TMA, to remove the autoantibodies against ADAMTS-13 and restore its activity. In contrast, TA-TMA is characterised by > 5% ADAMTS-13 activity, and is caused by a direct toxic effect, typically of the immunsuppressive drug. Therefore, in this condition, plasma exchange is not effective and treatment solely exists of withdrawal of the drug and supportive therapy.
This case illustrates the necessity of ADAMTS-13 testing in post-HSCT patients with TMA to exclude an underlying non-transplant-related aetiology.

P4.13 CMYB expression during in vitro hematopoiesis from human embryonic stem cells

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Introduction
The MYB proto oncogene a member of the family of myeloblastosis transcription factors. Abnormal regulation of this key hematopoietic transcription factor leads to leukemia including AML and T-ALL. In mice, yolk sac hematopoiesis is MYB independent and generates short lived blood cells during early fetal life and tissue macrophages such as Kupffer cells, microglia and Langerhans cells which persist during adult life, explaining lack of chimerism upon bone marrow transplantation. In contrast, hematopoietic stem cell based definitive hematopoiesis is MYB dependent.
Here, we unravel the role of MYB expression during human hematopoiesis from human embryonic stem cells (hESC), which recapitulates hematopoiesis during fetal life. For this study, reporter hESC lines (MYBeGFP-hESC) were generated carrying a BAC based cMYBeGFP reporter construct.

Results
cMYBeGFP-hESC were differentiated *in vitro* to blood cells using the spin embryoid body method. Hemogenic endothelium as well as the earliest $CD43^+CD34^+$ hematopoietic precursors were GFP negative, compatible with yolk sac-like hematopoieic activity. Analysis of kinetics during the formation of blood cells, $eGFP^+$ cells became apparant in the $CD34^+$ progenitor population at day11 of differentiation, forming a clear population by day14. These were included within both $CD45^-$ and $CD45^+$ fractions, which contain $CD123^+CD34^+$ common myeloid and granulocyte macrophage precursors, respectively. Both megakaryocytic and erythroid precursors showed a lack of eGFP expression.
In NK differentiation cultures, $CD117^+$NK precursors and GFP^-CD94^+ mature NK cells were $eGFP^-$. In some cultures, a population of $GFP^{bright}CD117^+CD94^-CD56^+CD161^+NKp44^+$ cells, phenotypically compatible with both NK precursors and innate lymphoid cells 3 (ILC3), was observed.
During induced myeloid lineage differentiation of $eGFP^-CD34^+CD45^+$progenitors, high level eGFP expression was observed during eosinophil and neutrophil differentiation, while $CD14^+$ monocyte differentiation showed no stage of apparent MYBeGFP expression, in line with cMYB independent hematopoiesis.

Conclusion
The data presented here provides understanding of the processes underlying malignant hematopoiesis during myeloid development, and provides a unique opportunity to study cMYB independent hematopoiesis *in vitro*.

P4.14 Autologous stem cell transplantation for late-onset nemaline myopathy and gammopathy

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Sporadic late-onset nemaline myopathy (SLONM) is an extremely rare disorder of undetermined etiology, occurring in adults over 40 years of age. It is characterised by progressive muscle weakness

that can variably affect proximal, distal or axial muscles, leading to severe disability or death. Diagnosis is confirmed by the detection of nemaline rod bodies in myofibers on muscle biopsy. When associated with a monoclonal gammopathy (MGUS), it carries a more unfavourable prognosis and usually fails to respond to immunosuppressive therapy. We report a case of SLONM with MGUS effectively treated with high dose chemotherapy and autologous stem cell transplantation (ASCT).
A 39-year-old woman without familial history of myopathy developed a progressive proximal upper extremity weakness with muscle atrophy, followed by lower extremity weakness. Routine laboratory tests were normal but identified an IgG kappa MGUS. HIV antibodies and inflammatory markers were negative. EMG and muscle biopsy were unremarkable.
During the next 2 years, her clinical status continues to worsen, as she developed severe proximal and axial weakness, making her unable to stand or walk unaided, and totally dependent for many daily life activities. She gradually developed dysphagia and required percutaneous gastrostomy for enteral feeding. A second muscle biopsy showed multiple rod bodies deposition in atrophic muscular fibers type I and II.
As she failed to respond to corticosteroids and plasmapheresis, she underwent high dose chemotherapy with melphalan 140 mg/m^2 followed by ASCT, after approval by the local ethics committee. A grade 4 febrile neutropenia with acute bacterial pneumonia required empirical large spectrum antibiotics. The patient was discharged on day 32, and attended a rehabilitation program. Three months after the procedure, no M-protein was detectable in the serum. The 7-month clinical follow-up showed an improvement in muscle strength and function, with disappearance of swallowing problems.
Although further follow-up is needed, our observation tends to confirm previous reports on the role of aggressive immunotherapy in SLONM associated with MGUS.

P4.15 Disseminated fusariosis following induction chemotherapy for acute leukemia

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Fusarium spp. are ubiquitous soil saprophytes and occasional plants pathogens. In immunologically competent hosts, fusariosis is usually limited and superficial. In contrast, patients with compromised immune functions are at high risk for invasive fusariosis, particularly in the setting of prolonged neutropenia.
We report the case of a 41-year-old woman diagnosed with acute myeloblastic leukemia, who developed at day +1 of the second induction course, high-grade fever with myalgia, with multiple tender, round, ulcerated papules and plaques scattered on the skin. Skin lesions appeared 7 days before the first positive blood sample, while the patient was under antifungal prophylaxis with posaconazole. Fusarium species was cultured from 10 blood samples, two days after the first positive histopathological skin biopsy. Empirical antifungal therapy with caspofungine was initiated at day +5, and replaced at day +11 by ambisome and voriconazole after fungal identification. Surgical drainage was required as abscesses developed with bone marrow regeneration. The patient was discharged on day +57 and remained on voriconazole alone. She underwent an allogeneic stem cell transplantation after a myeloablative conditioning from a familial donor without any recurrence of fungal infection.
Systemic fusariosis is associated with a high mortality in immunocompromised patients. Host immune status is the single most important factor predicting outcome. Optimal treatment has not yet been established.

P4.16 Pneumomediastinum as a complication of allogeneic bone marrow transplantation

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Invasive pulmonary aspergillosis (IPA) is a fungal infection frequently observed in immunocompromised patients. We present here a patient who developed pneumomediastinum following IPA during the course of refractory Hodgkin's lymphoma.
A 23-year-old man was referred for progressive dyspnea with severe hypoxemia, eight months after an unrelated allogeneic stem cell transplantation with reduced-intensity conditioning for a stage II mediastinal Hodgkin's lymphoma, refractory to multiple lines of therapy including chemotherapy, radiotherapy and autologous stem cell transplantation (ASCT). His treatment consisted in corticosteroids and tacrolimus for a cutaneous and digestive graft-versus-host disease (GVHD). Physical examination showed a poor performance status with severe denutrition, no fever, pulmonary crepitations and Sao2<88%. Blood tests were irrelevant except for a mild inflammation (CRP 1.6 mg/dl). Chest computed tomography identified bilateral diffuse alveolar infiltrates with multiple excavated nodules suggestive of invasive pulmonary aspergillosis, but also perivascular emphysema with pneumomediastinum and sign of tension around the left atrium. Bronchoalveolar lavage confirmed the presence of Aspergillus fumigatus. Despite large spectrum antibiotics and antimycotic therapy, the respiratory distress worsened and the patient died of respiratory failure.
This report deserves attention because (i) invasive pulmonary aspergillosis is a common complication in immunocompromised patients, (ii) and pneumomediastinum or pneumopericardium represent rare complications of IPA. These thoracic air-leakages are, in the majority of the cases, more indicative of the severity of the underlying pulmonary disease than a life-threatening entity by itself.

P4.17 Time To Neutrophils And Platelets Recovery In Patients Transplanted With Plerixafor Mobilized Hematopoietic Stem Cells, At The Institut Jules Bordet

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Introduction
This study reports a single centre experience of the outcome of patients proceeded to autologous transplantation with Plerixafor mobilized hematopoietic stem cells (HSC) between October 2011 and June 2013. The median engraftment times for neutrophils (ANC > 0.5 x 10^9/L) and platelets (> 20x10^9/L) have been analysed and compared to the results of patients auto-transplanted in 2012 with G-CSF mobilized HSC alone, at the Institut Jules Bordet.

Population
25 adult patients, older than 18 years have been reviewed: 9 in the plerixafor group: 6 women,3 men and 16 in the G-CSF alone group: 5 women, 11 men.

Methods
CD34+ transplanted cell dose, engraftment time for neutrophils as the first of 3 consecutive days recovery with absolute neutrophils count(ANC) > 0.5 x 10^9/L, for platelets recovery as the first day where the platelets > 20x10^9/L without transfusions were analysed.

Statistical analysis: using the non parametric Wilcoxon test for independent data; two-sided p values are reported .

Results
he median of CD34+ HSC infused was 3,4 (2,00-4,30) × 10^6/kg in the plerixafor group and 3,6 (3,1-5,1) × 10^6/kg in the G-CSF alone group. No statistically significant differences could be detected for CD34+HSC (p=0.39) between the two groups. In both groups there were no patients who do not recovered. The median engraftment times for neutrophils were 10 (9-16) days and platelets were 20 (10-32) days in the Plerixafor group compared to 12 (9-15) days for neutrophils and 14 (0-22) days for platelets in the G-CSF alone group . No statistically significant differences could be detected for ANC (p=0.80) and for platelets (p=0.06). Autologous transplantation with Plerixafor mobilized HSC was well tolerated and adverse events were very similar to G-CSF HSC transplantation.

Conclusion
Our observation confirms that Plerixafor is a good alternative for poor mobilizer patients. Moreover, the outcome of patients transplanted with Plerixafor mobilized grafts is very similar to those transplanted with G-CSF mobilized grafts in terms of time to reach a sustained neutrophils and platelets recovery.

P4.18 A long (GT)n repeat genetic variant in the promoter region of heme oxygenase-1 is associated with severe graft-versus-host disease

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Allogeneic stem cell transplantation is an important therapeutic option for various malignant and non- malignant diseases. However, the development of graft-versus-host disease (GVHD) limits the success of transplantation and represents a leading cause of early mortality. Heme oxygenase-1 (HO-1) is a stress-inducible enzyme with anti-inflammatory and cytoprotective properties. Upregulation of HO-1 activity has been shown to protect mice from acute GVHD after allogeneic bone marrow transplantation. A functional (GT)n repeat variant in the HO-1 promoter region is associated with HO-1 protein expression (i.e. short (<30) (GT)n repeat carriers present increased HO-1 expression). Therefore, we have conducted a genetic association study of HO-1 promoter (GT)n repeat variants in bone marrow transplanted patients.

We retrospectively analyzed patients undergoing allogeneic stem cell transplantation in Jules Bordet Institute between 2001 and 2011. Length polymorphism was analysed in 160 donor-host pairs and 203 healthy controls. For genotypic frequency analysis, the number of GT repeats were grouped into short (<30 GT repeats) and long (≥ 30 GT repeats) alleles. Using χ^2 test, we confirmed that donor, host and control allele distributions were identical. Therefore, genotype distribution of homozygous long (GT)n profiles (LL), homozygous short (GT)n profiles (SS) and heterozygous long/short (GT)n profiles (LS) was analysed. We found that in severe acute GVHD the proportion of S allele carrier in the donor was significantly lower (p=0,04). These findings indicate in a representative European cohort of bone marrow transplanted patients, that donor polymorphism leading to low expression of HO-1 is associated with increased GVHD severity. Although recipient HO-1 gene polymorphisms suggested a similar effect, this did not reach statistical significance. We conclude that HO-1 polymorphism could represent a new genetic risk factor for GVHD severity and that the induction of HO-1 activity could be an interesting therapeutic approach to reduce GVHD. Confirmation of these observations in an independent cohort is ongoing.

P4.19 Extracorporeal photopheresis for treatment in hematological diseases : a single centre experience

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Background
Extracorporeal photopheresis (ECP) has emerged as an effective approach for the management of resistant graft versus host disease (GVHD) acute(a) or chronic(c) and cutaneous T cell lymphoma (CTCL). Here, we report the results of ECP in children and adults with the 'off-line' approach in our centre.

Population
Four adults were treated for CTCL: 3 with advanced-stage and 1 with Mycosis Fungoides(MF). Four children and 7 adults were treated for GVHD: 3 children with steroid refractory grade II- IV gastrointestinal and cutaneous aGVHD; 7adults and 3 children with limited or extensive cGVHD.

Methods
For CTCL, ECP was performed on 2 consecutive days monthly during the first 3 months (mos). For cGVHD the patients received on 2 consecutive days every two weeks during the first 3 mos. According to the clinical response, ECP reduced at one cycle every 4 weeks for the next 3 mos. Concerning aGVHD, ECP was administered three times per week until complete clinical response, then 2 consecutive days per week , then biweekly and finally monthly.

Results
Median time to start ECP for CTCL after diagnosis was 8 (1/2-14) years: two patients showed no response after 6 mos and switched to other treatments. One patient was stabilized during 18 mos, then became refractory. The patient with MF is still under treatment with partial response at 3 mos. Concerning aGVHD: one patient archived completed response after 7 mos, one after 1month but relapsed with cGVHD of the skin 6 mos after and another patient with partial response after 1 mos, developed a skin cGVHD. For cGVHD, median time to start ECP after cGVHD diagnosis was 33 (6-132) months. Median number of treatments was 17 (9-45) courses and duration of treatment = 4 (2-9) mos. Two adult patients died during treatment and 8 patients are still under treatment.

Conclusion
Preliminary results of our small serie confirm that ECP with the the 'off-line' approach is a safe and effective treatment in children and adult with CTCL and GVHD.

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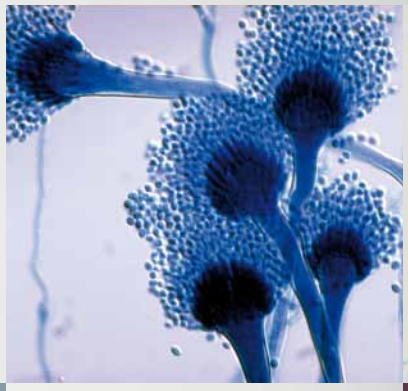




NAME OF THE MEDICINAL PRODUCT AmBisome 50 mg powder for concentrate for solution for infusion **QUALITATIVE AND QUANTITATIVE COMPOSITION** Each vial contains as active ingredient 50 mg of amphotericin B (50,000 units) encapsulated in liposomes. After reconstitution, the concentrate contains 4 mg/mL amphotericin B **PHARMACEUTICAL FORM** Powder for concentrate for solution for infusion. AmBisome is a sterile, powder for solution for infusion. AmBisome is a yellow lyophilised cake or powder. After reconstitution with water for injections the concentrate is a translucent, yellow dispersion. **THERAPEUTIC INDICATIONS** AmBisome is intended for the treatment of serious systemic and/or deep fungal infections in patients who do not react to conventional amphotericin B or patients in whom administration is contraindicated due to kidney problems. AmBisome is also indicated for treatment of suspected fungal infections in febrile neutropenic patients. Fungal infections which have been successfully treated with AmBisome include diffuse candidiasis, aspergillus and mucormycosis infections, chronic mycetoma and cryptococcic meningitis. AmBisome should also be effective in the treatment of the following fungal infections: North American blastomycosis, coccidiomycosis, histoplasmosis, mucormycosis caused by particular sorts of Mucor, Rhizopus, Absidia, Entomophthora, Basidiobolus and sporotrichosis caused by Sporothrix schenckii. This drug may not be used for the treatment of trivial, clinically undistinguished fungal infections which only show up on skin or serological tests. **POSOLOGY** Adults: AmBisome is administered by intravenous infusion over a period of 30 to 60 minutes. The recommended concentration is 0.2 mg/mL to 2.0 mg/mL amphotericin B as AmBisome. The dose must be adapted to the specific requirements of each patient. Treatment is usually started with a daily dose of 1 mg/kg body weight, and can, if wished, gradually be increased to 3 mg/kg body weight. The recommended dose for treatment of suspected fungal infections in febrile neutropenic patients is 3 mg/kg/day. An average treatment consists of a stepwise increasing cumulative dose of 1 to 3 g amphotericin B as AmBisome given over a period of 3-4 weeks. Paediatric Patients Systemic fungal infections have been successfully treated with AmBisome in paediatric patients without reports of unusual adverse events. Paediatric patients have received AmBisome at doses comparable to those used in adults on a per kilogram body weight basis. Elderly Patients There are insufficient data available on the treatment of elderly patients to provide specific recommendations for these patients. There are however no data to suggest that elderly patients should be dosed differently. Renal Impairment AmBisome has been successfully administered to a large number of patients with pre-existing renal impairment at starting doses ranging from 1-3 mg/kg/day in clinical trials and no adjustment in dose or frequency of administration was required. **CONTRAINDICATIONS** AmBisome is contraindicated in those patients who have shown hypersensitivity to any of its constituents. **ADVERSE REACTIONS** In general, adverse effects of AmBisome have been similar to those of conventional amphotericin B, but are less frequent and less severe. Fevers and chills/rigors are the most frequent infusion-related reactions expected to occur during the first AmBisome dose administration when no premedication to prevent these reactions is provided. In two doubleblind, comparative studies, AmBisome treated patients experienced a significantly lower incidence of infusion-related reactions, as compared to patients treated with conventional amphotericin B or amphotericin B lipid complex. Less frequent infusion-related reactions may consist of one or more of the following symptoms including back pain and/or chest tightness or pain, dyspnoea, bronchospasm, flushing, tachycardia, and hypotension, and these resolved rapidly when the infusion was stopped. These reactions may not occur with every subsequent dose or when slower infusion rates (over 2 hours) are used (see Special Warnings and Precautions for Use on prevention or treatment of these reactions). Nephrotoxicity occurs to some degree with conventional amphotericin B, in most patients receiving the drug intravenously. In two, double-blind studies, the incidence of nephrotoxicity with AmBisome (as measured by serum creatinine increase greater than 2.0 times baseline measurement), is approximately half of that reported for conventional amphotericin B or amphotericin B lipid complex. The following adverse reactions have been attributed to AmBisome. The incidence is based on analysis from pooled clinical trials of 688 AmBisome treated patients. The adverse reactions are listed below by body system organ class using MedDRA and are sorted by frequency. Frequencies are defined as: Very common ≥ 10% Common ≥ 1% and < 10% Uncommon ≥ 0.1 % and < 1% BLOOD AND LYMPHATIC SYSTEM DISORDERS Uncommon:Thrombocytopenia IMMUNE SYSTEM DISORDERS Uncommon: Anaphylactoid Reaction METABOLISM AND NUTRITION DISORDERS Very common: Hypokalemia Common:hypomagnasemia, hypocalcemia, hyperglycemia, hyponatremia NERVOUS SYSTEM DISORDERS Common: headache Uncommon: convulsion CARDIAC DISORDERS Common: tachycardia VASCULAR DISORDERS Common: vasodilatation, flushing, hypotension RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS Common: dyspnoea Uncommon: bronchospasm GASTROINTESTINAL DISORDERS Very common: nausea,vomiting Common: diarrhoea, abdominal pain HEPATOBILIARY DISORDERS Common: Liver function tests abnormal (hyperbilirubinemia, alkaline phosphatase increased, elevated transaminases) SKIN AND SUBCUTANEOUS DISORDERS Common: rash MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS Common: back pain GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS Very Common: pyrexia, rigors Common: chest pain RENAL AND URINARY DISORDERS Common: increased creatinine, blood urea increased, In addition to adverse reaction reports from clinical trials, the following possible adverse reactions have also been identified during post-marketing use of AmBisome. VASCULAR DISORDERS Frequency not known: hypertension IMMUNE SYSTEM DISORDERS Frequency not known: anaphylactic reactions, hypersensitivity SKIN AND SUBCUTANEOUS DISORDERS Frequency not known: angioneurotic oedema RENAL AND URINARY DISORDERS Frequency not known: renal failure, renal insufficiency Impaired renal function is common in patients receiving conventional amphotericin B. The glomerular filtration rate almost always decreases (by up to 40%) at the start of treatment. It remains lowered over the entire period of treatment in most patients. There is an increase in blood levels of substances usually eliminated with the urine, such as creatinine and urea. Permanent renal impairment beyond the end of treatment is occasionally observed. Hypokalemia due to renal acidosis may occur in around 20% of patients. Anaemia is common in patients receiving conventional amphotericin B. Blood count changes are generally reversed after termination of the treatment. Temporary loss of hearing, tinnitus, visual disorders and double vision have been observed in rare cases during treatment with conventional amphotericin B. Hypertension, hypotension, cardiac arrhythmias and cardiac arrest have occurred following infusion of conventional amphotericin B in individual cases. **MARKETING AUTHORISATION HOLDER** Gilead Sciences International Limited Cambridge CB21 6GT United Kingdom **MARKETING AUTHORISATION NUMBER(S)** BE166257 **DATE OF REVISION OF THE TEXT** 01/2014 **MODE OF DELIVERY** Prescription only **PRICE** 1063,14 EUR (10 vials)

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