An increased cytokine response may be driving early cytopenia in myelodysplastic syndromes

Nielsen, A. B., Salem, M., Hansen, J. W., Ørskov, A. D., Bruunsgaard, H. and Grønbæk, K.

Introduction: Myelodysplastic syndromes (MDS) are clonal neoplasms originating in bone marrow stem cells, leading to ineffective hematopoiesis. Deregulation of innate immune and inflammatory signalling has been suggested to cause a bone marrow microenvironment that is permissive for the pathogenesis and the progression of MDS.

Methods: We assessed whether blood plasma concentrations of 19 inflammatory markers differed between three groups of cytopenic patients (idiopathic cytopenia of unknown significance (ICUS) N=40, clonal cytopenia of undetermined significance (CCUS) N=31, and MDS, low-risk N=20, high-risk N=19) and 21 age-matched healthy control subjects. Plasma was collected between 2014 and 2018 at Department of Hematology, Rigshospitalet, Denmark, and analyzed using luminex multiplex cytokine screening and enzyme linked immunosorbent assay. The inflammatory markers included were IL-1-ra, IL-1α, IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL17A, TPO, VEGF-A, GM-CSF, S100A9, CXCL10, RANTES, IFN-γ, TNF-α and TGF-β1. Through linkage with Danish nationwide registers, we also assessed whether cytokines were associated with previously demonstrated risk factors for MDS including mutational status and blood counts.

Results: Patients had statistically, significantly lower blood plasma concentrations of active TGF-β1 and RANTES, and higher concentrations of IL-6, IL-7, IL-10, CXCL10 and TNF-α compared to controls. IL-10 levels were furthermore increasing between ICUS and CCUS patients and CCUS and MDS patients. Additionally, the IL-6 blood plasma concentration was associated with having a mutation (Odds Ratio 0.87, 95%CI: 0.76-0.97, p=0.02)

Conclusions: Our findings suggest that an increased cytokine response is involved in the pathogenesis of cytopenia, since cytokine levels differed between cases and healthy controls, but not between patient groups. IL-10 may enhance risk of progression from cytopenia to MDS. Furthermore IL-6 was associated with mutational status.

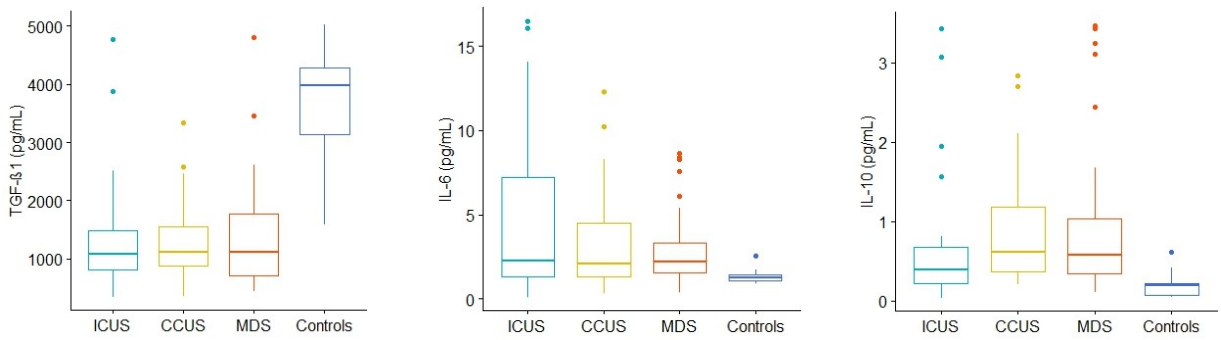


Figure 1-3: Blood plasma concentration of cytokines within groups of patients with ICUS, CCUS and MDS and age matched healthy controls.

Identifying Newly Diagnosed Patients with Chronic Lymphocytic Leukemia without Need of Treatment or Specialized Care

Christian Brieghel, Rudi Agius, Caspar da Cunha-Bang, Michael Asger Andersen, and Carsten Utoft Niemann

CLL laboratory, Department of Hematology, Rigshospitalet.

Despite an indolent disease course in up to half of patients with chronic lymphocytic leukemia (CLL), majority of prognostic indices focus on adverse outcome. This study aimed to predict indolent CLL.

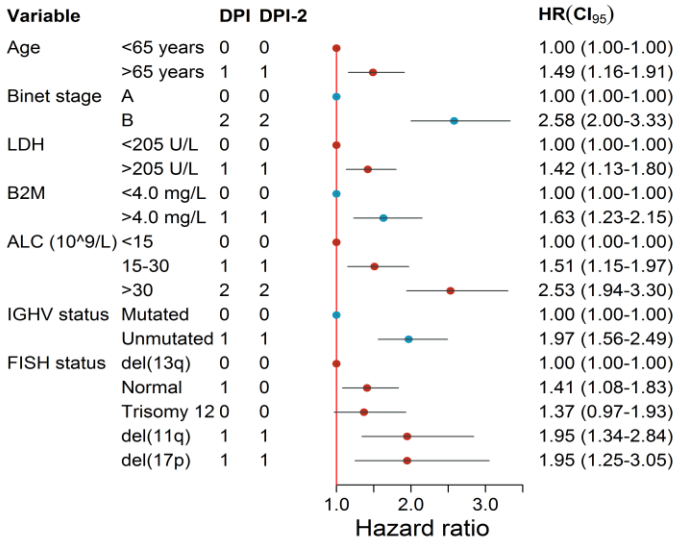
We included 4120 newly diagnosed patients from the Danish CLL registry. Data on age, Binet stage, beta-2-microglobulin, IGHV status, FISH status (CLL-IPI) and gender were added with LDH and absolute lymphocyte counts (ALC) from PERSIMUNE. A training set (80%) and test set (20%) were randomly sampled. As the only IWCLL treatment criterium, patients diagnosed with Binet stage C were excluded. Endpoints were treatment-free survival (TFS) and time to first treatment (TTFT) considering death as a competing risk.

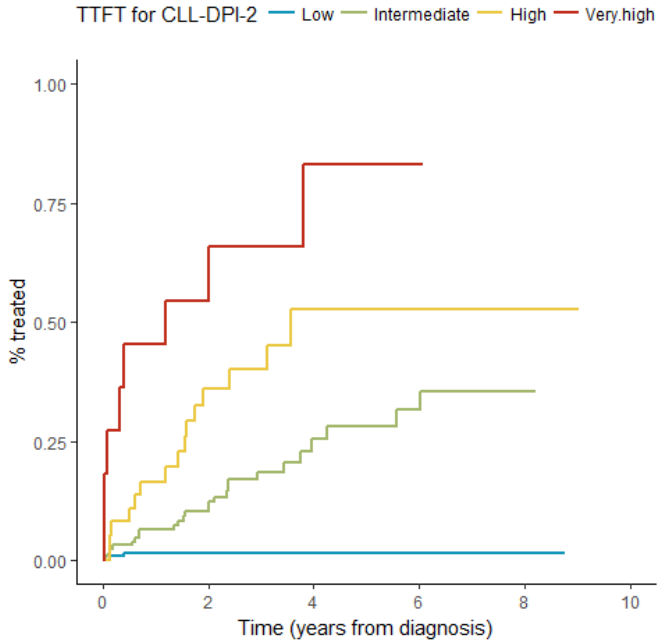
With a median follow-up of 4.5 years, 886 patients (27%) had received first line treatment and 452 (14%) had died without receiving treatment. In a multivariable Cox model with backward elimination, an independent association with poor TFS was demonstrated for all variables, except for gender. Weighting independent risk factors to create a Danish prognostic index (CLL-DPI), composite scores 0-1, 2-3, 4-5, and 6-10 points defined risk groups low, intermediate, high and very high risk, respectively (Figure 1).

Internally validating the index in the test set, 81 (27%) patients with CLL-DPI low risk demonstrated 96% 5-year TFS and 0% received treatment.

Assigning normal FISH to 0 points, 125 (42%) patients were CLL-DPI-2 low risk with 90% 5-year TFS and only 1.6% receiving treatment (Figure 2). Proportion of patients with CLL-IPI low risk was considerably larger (61%) but demonstrated 76% 5-year TFS and 9% were treated within 5 years.

With less than 2% of patients requiring treatment within 5 years from diagnosis, CLL-DPI-2 may allow for safe discharge of patients with newly diagnosed CLL, potentially improving quality of life, while at the same time prioritizing spare specialized hematology services for patients at higher risk.



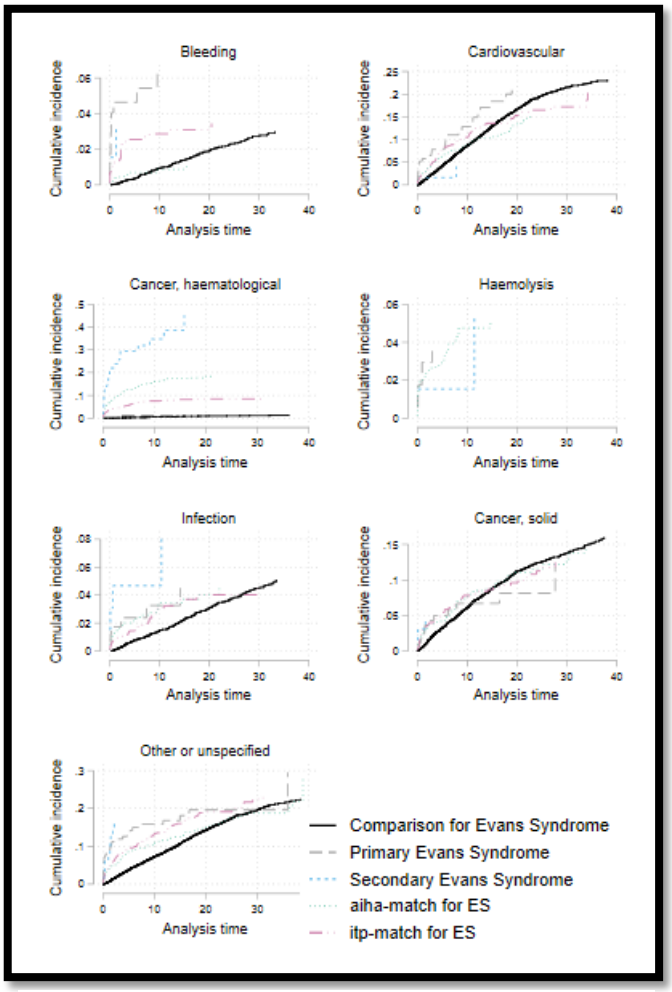


Forfattere: Dennis Lund Hansen[1], Sören Möller[2], Kjeld Andersen[3], David Gaist[4] og Henrik Frederiksen[1]
1: Hæmatologisk afdeling X, Odense Universitetshospital, 2: OPEN, Odense Universitetshospital, 3: Psykiatrisk Afdeling Odense – Universitetsfunktion, 4: Neurologisk afdeling, Odense Universitetshospital

Overlevelse og dødsårsager ved Evans Syndrom, DHS 2019

Den Danske Hæmolysekohorte er en landsdækkende retrospektivt defineret kohorte, indeholdende alle patienter med hæmolyse eller primær immuntrombocytopeni (ITP) diagnose fra 1977 til 2017. Kohorten baserer sig på sammenkørsel af en række danske sundhedsregistre, og et forudgående valideringsstudie af 400 patienter med hæmolysesygdomme. Kohorten rummer 242 patienter der har fået Evans Syndrom efter de er fyldt 13 år.

Vi har tidligere beskrevet, hvordan disse patienter er identificeret og at de har en markant nedsat overlevelse. I dette studie viser vi, at Evans Syndrom ikke alene er associeret med nedsat overlevelse i forhold til de definerende enkeltsygdomme immuntrombocytopeni (ITP) og autoimmun hæmolytisk anæmi (AIHA), men også at dødårsagerne fordeler sig anderledes. Vi har undersøgt syv klasser af dødsårsager: blødning, cardiovaskulære, hæmatologisk cancer, hæmolyse, infektion, solid cancer og ”andre”.



Figur 1: Kumuleret mortalitet blandt Primær Evans Syndrom og Sekundær Evans Syndrom, sammenlignet med kontroller fra baggrundbefolkningen samt matchede patienter med AIHA og ITP.

Beregninger på konkurrenden dødsårsager viser at Evans Syndrom medfører signifikant øget risiko for blødnings-, hæmolyse- og infektionsrelateret død, både i forhold til befolkningen som helhed og i forhold til alders- og kønsmatchede patienter med AIHA og ITP.

Cause-specific proportional hazard regression viser at alle undersøgte dødsårsager, på nær solid cancer, har øget dødsrate blandt patienter med Evans Syndrom sammenlignet med baggrundsbefolkningen.

Preliminary Data from Protocol MPN1801: A Phase-1-First in Man Study in Patients with Calreticulin-Mutant Myeloproliferative Neoplasms by vaccinating with Calreticulin Exon 9 Mutant Peptide.

Jacob Handlos Grauslund1,2, Morten Orebo Holmström1,2, Stine Emilie Weis-Banke2, Uffe Klausen2, Nicolai grønne Jørgensen 2, Daniel El-Fassi3, Mette Borg3, Lasse Kjær1, Vibe Skov1, Inge Marie Svane2,4, Hans Carl Hasselbalch1, Mads Hald Andersen2, 5
1 Department of Hematology, Zealand University Hospital
2 Center for Cancer Immune Therapy, Department of Hematology, Copenhagen University Hospital Herlev
3 Department of Hematology, Copenhagen University Hospital Herlev
4 Department of Oncology, Copenhagen University Hospital Herlev
5 Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

Background:

The calreticulin (*CALR*) exon 9 mutations are widely immunogenic both in patients with *CALR*-mutant myeloproliferative neoplasms (MPN) and in healthy donors, and CALR-mutation specific T-cells recognize autologous *CALR*-mutant cells from both peripheral blood and bone marrow. Accordingly, therapeutic cancer vaccination against the *CALR*-mutations is an intriguing treatment option to investigate.

Aim:

To test the safety and toxicity of CALR-mutant peptide vaccination in *CALR*-mutant MPN patients. Changes in immune phenotype and immune reactivity and clinical response will be assessed as well.

Methods:

Ten CALR-mutant MPN patients will receive 15 vaccinations with the CALRLong36 epitope with Montanide ISA-51 as an adjuvant (**Figure 1**).
Adverse events will be evaluated according to CTCAE, changes in immune phenotype and immune reactivity will be evaluated by ELISPOT and FACS. Clinical response is evaluated by peripheral blood analysis, bone marrow examinations and the *CALR*-mutant allele burden. Bystander mutations will be described using Next Generation Sequencing (NGS).

Results:

As of January 31, 2019, nine patients have received between 1 and 7 vaccines – a total of 37 vaccines have been administered.
AE have been mild with only grade 1-2 AEs. AEs have been both local and systemic.
Some patients have displayed a modest decrease in platelet counts during the first months of vaccinations.
One patient (no. 3), a 62-year-old male with PMF treated concurrently with interferon-alpha and the vaccines, displayed a drop in *CALR*-mutant allele burden from 38% to 31% after 6 vaccines. In the same period of time, the patient displayed a decrease in platelet counts from 145x10^9/l to 107x10^9/l (**Figure 2**).

Conclusion:

After 37 administered vaccines, the vaccination is well tolerated. No conclusions on neither the clinical nor the immunological effect can be made yet. The vaccine does not seem to induce disease progression/transformation.

	Inclusion	Study period													End of study
Visit		1	2	3	4	5	6	7	8	9	10		15	16
Week	-4 to 0	1	3	5	7	9	11	15	19	23	27	...	47	48
Vaccine CALRlong36		V	V	V	V	V	V	V	V	V	V	...	V	
Health blood sample[a]	HB	HB	HB	HB	HB	HB	HB	HB	HB	HB	HB	...	HB	HB
Study blood sample[b]	SB x 2				SB			SB				...		SB
Physical Examination	PE	PE	PE	PE		PE		PE			PE	...		PE
Electrocardiography	ECG	ECG	ECG	ECG		ECG		ECG			ECG	...		ECG
Bone marrow aspiration	BM											...		BM
Delayed type hypersensitivity[c]								DTH				...		
Next generation sequencing	NGS													NGS
Adverse Events	AE	AE	AE	AE	AE	AE	AE	AE	AE	AE	AE	...	AE	AE

Figure 1. Treatment Schedule

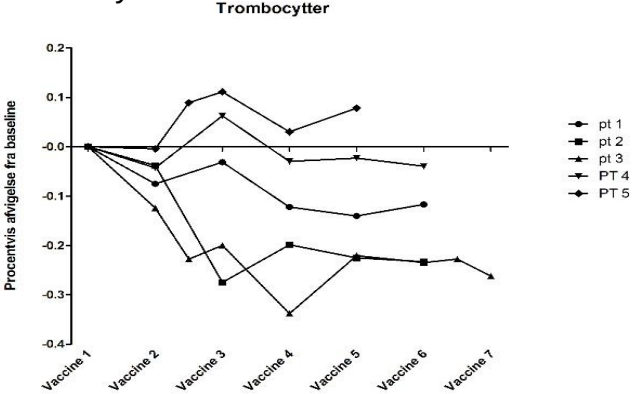


Figure 2. Relative change in platelet counts during vaccines.

Osteoporosis and Low-Energy Fractures After Treatment for Lymphoma with Corticosteroid-Containing Immunochemotherapy: A Danish Nationwide Cohort Study

Introduction

Commonly used immunochemotherapy regimens for large B-cell lymphomas (DLBCL) and follicular lymphomas (FL) contain repeated short cycles of prednisolone for up to 8 cycles, resulting in cumulative dosage of up to 4000 mg or an average dosage of 24 mg/day over 24 weeks (8 x R-CHOP-21). We investigated the risk glucocorticoid-induced osteoporosis (GIO) (including low-energy fractures) in lymphoma patients treated with glucocorticoid-containing immunochemotherapy (GCI) and compared findings to a matched background population.

Methods

Adult patients diagnosed with DLBCL and FL between 2000-2012 and treated with R-CHOP, R-CHOEP, or R-CVP were retrieved from the Danish Lymphoma Registry (LYFO). Patients with previous osteoporosis were excluded. A background population was constructed by matching 5 random persons from the Danish Civil Registry per patient matched on sex, month/year of birth and without previous osteoporosis. Incident osteoporosis were identified from Danish National Patient Registry. Prescriptions of bisphosphonates or denosumab according to the Danish National Prescription Registry were also used as proxies for osteoporosis. Follow-up was from completion of first-line chemotherapy to osteoporosis or the end of the study in December 2014. Univariate- and multivariable Cox regressions were performed to assess the relationship between GCI and osteoporosis.

Results

2,780 patients were matched to 13,900 random Danish citizens. Median follow-up was 5.8 and 6.0 years for patients and controls. The unadjusted hazard ratio (HR) for osteoporosis for lymphoma patients versus matched controls was 1.50 [1.31;1.72] (Table 1). Patients treated with GCI had 1-, 5-, 10-year cumulative risks of osteoporosis at 4%, 12%, and 18%, whereas risks for controls were 2%, 8%, and 15% (Fig. 1).

Conclusion

Lymphoma patients treated with GCI face substantially increased risk of osteoporosis. Female and elderly patients suffering comorbidities appears to be at particularly high risk and may benefit targeted screening for osteoporosis or bisphosphonate prophylaxis during lymphoma treatment.

Variable	Units	Univariate HR	CI.95	p-value	Multivariate HR	CI.95	p-value
Sex	Female	3.20	[2.84;3.60]	<0.01	3.08	[2.75;3.45]	<0.01
Age	Pr. year	1.05	[1.05;1.06]	<0.01	1.05	[1.05;1.06]	<0.01
Prednisolon		1.50	[1.31;1.72]	<0.01	1.50	[1.29;1.75]	<0.01
Liver Disease		2.94	[1.93;4.48]	<0.01	2.77	[1.78;4.30]	<0.01
Kidney Disease		2.26	[1.64;3.11]	<0.01	1.86	[1.33;2.58]	<0.01
Neoplasma Prostata		1.91	[1.22;3.00]	<0.01	2.29	[1.43;3.67]	<0.01
Breast Cancer		3.16	[2.41;4.14]	<0.01	1.57	[1.18;2.09]	<0.01
Thyroid Disease		1.67	[1.30;2.15]	<0.01	0.98	[0.75;1.28]	0.89
CCI*	0	Ref			Ref		
	1	2.04	[1.64;2.54]	<0.01	1.54	[1.23;1.93]	<0.01
	2	1.57	[1.26;1.95]	<0.01	1.01	[0.79;1.29]	0.92

Table 1. Univariate and multivariable Cox regression for the whole cohorte with p-values and 95% confidence intervals.
*CCI = Charlson Comorbidity Index, CI.95 = 95% Confidence Interval, HR = Hazard Ratio, Ref = Reference.

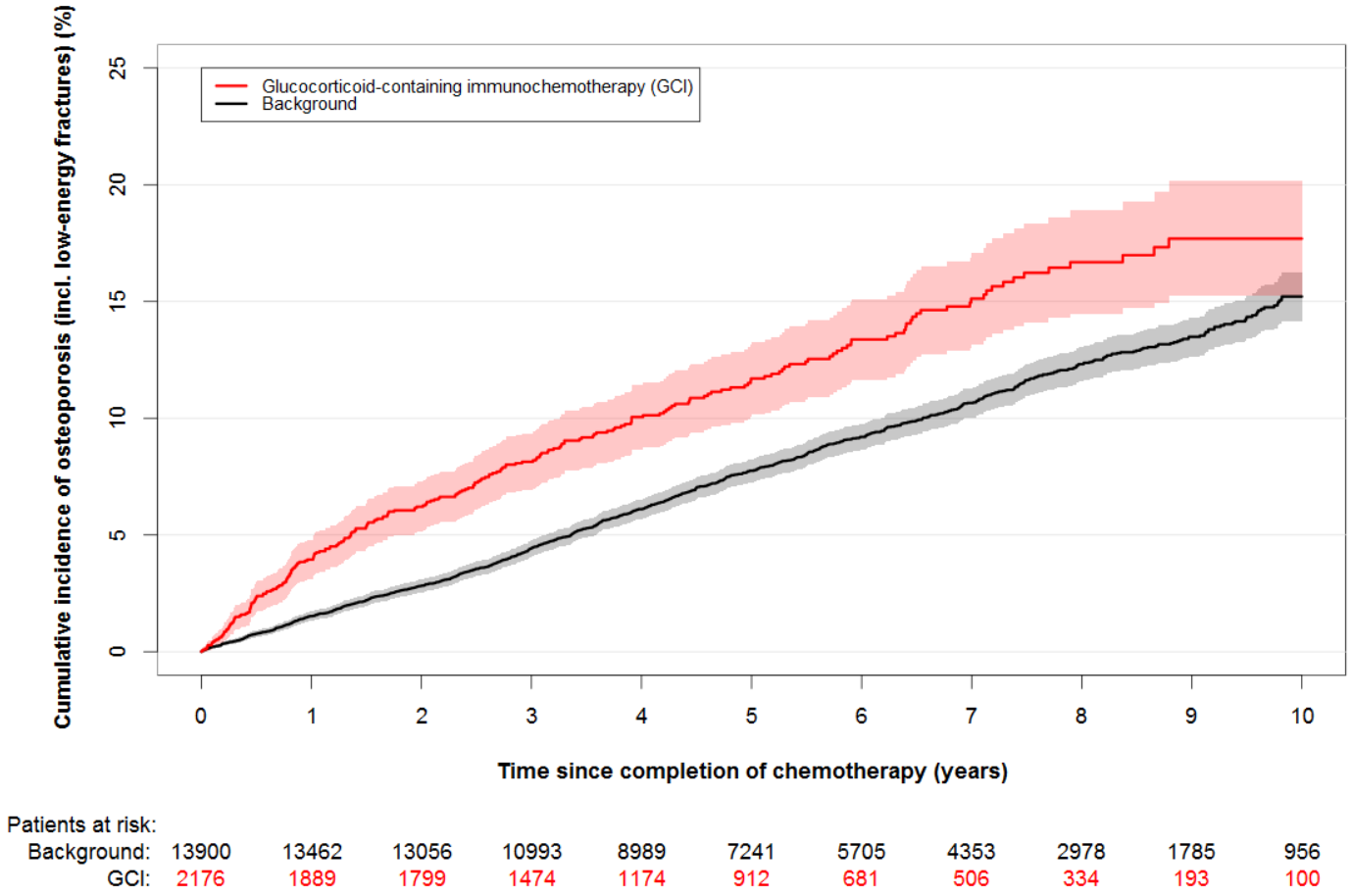


Figure 1. Risk of osteoporosis (including low-impact fractures) with all-cause mortality as competing risk.

TERT promoter methylation as a potential prognostic biomarker for progression of unexplained cytopenia to MDS and AML

K.Kaastrup[1,2], L.Gillberg[1,2], D.Aslan[1,2], L.S. Kristensen[1], A.D.Ørskov[1,2], J.W.Hansen[1,2,3] and K.Grønbæk[1,2,3]

[1]Dept. of Hematology, Rigshospitalet. [2]Biotech Research and Innovation Centre (BRIC), University of Copenhagen. [3]The Danish Stem Cell Center (Danstem), University of Copenhagen, Denmark.

Objectives

Patients with myelodysplastic syndrome (MDS) often have mutations in epigenetic regulators and altered DNA methylation in their blood cells. Patients with unexplained cytopenia that do not fulfill the criteria for MDS are referred to as having idiopathic cytopenia of undetermined significance (ICUS) or, if they have MDS-related mutations, cytopenia of undetermined significance (CCUS).

A previous study showed that acute myeloid leukemia (AML) patients, and even more AML patients with preceding MDS (MDS/AML), have hypermethylation of the telomerase reverse transcriptase (*TERT)* promoter, which may lead to impaired telomerase activity and play a role in the progression to AML.

Here, we investigated DNA methylation of the *TERT* promoter in 185 patients with ICUS, CCUS or MDS and 15 healthy controls, to evaluate its potential as a biomarker of disease severity in patients with unexplained cytopenia and MDS.

Methods

TERT promoter methylation of two regions (Figure 1) was analyzed by pyrosequencing. Results are presented as DNA methylation percentage (mean ± SD).

Results

TERT promoter methylation in region 1 was significantly higher in all disease groups compared to healthy controls, with increasing significance levels with increased disease severity (ICUS: 8.3±4.8, p=0.03; CCUS: 10.2±7.2, p=0.0007, MDS: 11.3±7.7, $p=1.9\times10^{-6}$).

In region 2, MDS patients had significantly higher methylation (MDS: 8.8±8.1, p=0.001) compared to healthy controls (5.4±3.2). DNA methylation of region 2 in ICUS and CCUS patients did not significantly differ from that of healthy controls.

Conclusion

Promoter DNA methylation of *TERT* increases gradually from healthy controls to ICUS, CCUS and MDS. This is in line with a previous study of MDS/AML patients. Our study indicates that DNA methylation of the *TERT* promoter reflects disease severity and is a potential biomarker for progression to high-risk MDS and AML.

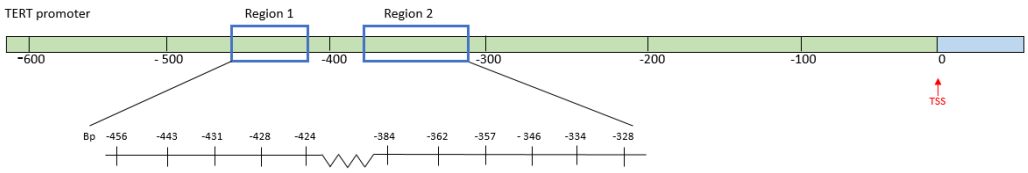


Figure 1: *TERT* promoter methylation levels was examined using two assays (region 1 and region 2) covering 11 CpG sites.

Daratumumab for treatment of blastic plasmacytoid dendritic cell neoplasm

A single-case report

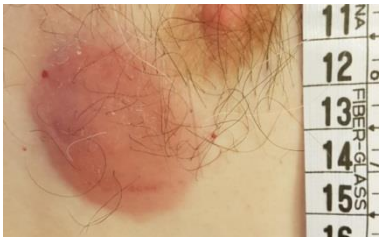
Katrine F Iversen[1], Paw C Holdgaard[2], Birgitte Preiss[3], Charlotte G Nyvold[4] and Torben Plesner[1]. [1]Department of Hematology, Vejle Hospital, Vejle, Denmark and University of Southern Denmark[2] Department of Nuclear Medicine, Vejle Hospital, Vejle, Denmark. [3]Department of Pathology, Odense University Hospital, Odense, Denmark. [4]Haematology-Pathology Research Laboratory, Department of Hematology, Odense University Hospital, Odense, Denmark.

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare myeloid malignancy with a poor prognosis. Median overall survival is 10-19.8 months.[1] The optimal treatment is considered to be induction chemotherapy as for acute lymphocytic leukemia followed by allogeneic stem cell transplantation (allo-SCT), but many elderly patients are not eligible for this intensive therapy.[2,3]

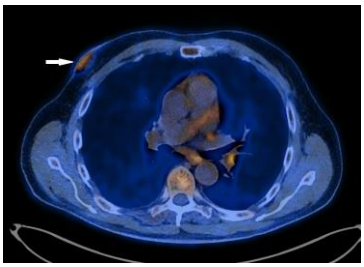
A 70-year-old male presented with a cyanotic, elevated, cutaneous element (Fig. 1A). By immunohistochemistry the cells were positive for CD4, CD56, CD123 and TCL1 but CD38 could not be detected. There was involvement of bone marrow, lymph nodes, spleen and skin (Fig 1B). Flow cytometry of the bone marrow aspirate revealed 4 % neoplastic plasmacytoid dendritic cells positive for CD4, CD56, CD123, CD303, TCL1 and CD38. Because of his age, the patient was ineligible for intensive therapy and allo-SCT.

An initial course of single-agent daratumumab was given to test its potential activity in BPDCN. Already after the first cycle, the fraction of neoplastic plasmacytoid dendritic cells in the bone marrow was decreased and the focal skin lesion was reduced in size (Fig. 1C). Subsequently the treatment was intensified as pre-planned with liposomal doxorubicin, bortezomib, azacytidine, lenalidomide and dexamethasone in combination with daratumumab for six 3-weeks cycles resulting in a complete response (CR). Maintenance with daratumumab was continued for additional 6 cycles.

Six months after the last dose of daratumumab the patient relapsed with constitutional symptoms and leukemia. Treatment with daratumumab, bortezomib, pomalidomide and dexamethasone was initiated. After 5 cycles of therapy evaluation revealed CR. The OS is now 23 months.



1A


1B

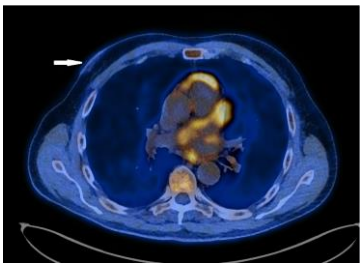

1C

Figure legends

Figure 1. The skin lesion. (A) Clinical photo. (B) Fused ^{18}F-FDG PET/CT images of the focal skin lesion before the initial treatment with single-agent daratumumab. The focal skin lesion (white arrow) had moderately increased FDG before treatment with a maximum standard uptake value (SUVmax) of 3.2. (C) After the initial treatment the FDG uptake had normalised with a SUVmax of 1.5 and the lesion had reduced in thickness. The patient also had a FDG positive lymph node of the neck (not displayed here) which also reduced in SUVmax from 18.9 to 14.5 and in size from 2.2 x 1.4 cm to 1.7 x 1.0 cm.

References

1. Swerdlow SH, Campo E, Harris NL, et al. WHO classification of tumors of hematopoietic and lymphoid tissues, Revised 4th edn, volume 2. 2016. IARC Press, Lyon.
2. Kim MJM, Nasr A, Kabir B, et al. Pediatric blastic plasmacytoid dendritc cell neoplasm: A systematic literature review. *J Pediatr Hematol Oncol*. 2017;39:528-537.
3. Il, DK, Sokol L. The advances in therapy of balstic plasmacytoid dendritic cell neoplasm. *Expert Opin Investig drugs*. 2018;27:733-739.

Long-term outcomes after allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with non-myeloablative and myeloablative conditioning: a single-center cohort study of 438 consecutive patients

Lars K. Gjærde, Niels S. Andersen, Lone S. Friis, Brian Kornblit, Søren L. Petersen, Ida Schjødt, Henrik Sengeløv

Department of Hematology, Rigshospitalet

Background: Since 2000, a non-myeloablative (NMA) conditioning regimen has been used for older (>50 years) or significantly comorbid younger patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) for acute myeloid leukemia (AML) at our institution. We aimed to compare the long-term outcomes in NMA versus myeloablative (MA) conditioned patients.

Methods: We included 220 NMA and 218 MA conditioned adult patients receiving their first allo-HSCT for AML from 2000-2017 at Rigshospitalet in a retrospective cohort. Transplant-related outcomes were compared using Gray's test.

Results: NMA and MA conditioned patients differed when regarding age (median 60 versus 42 years, respectively), Karnofsky score (<90 in 18% and 11%, respectively), stage at transplant (1st complete remission in 68% and 49%, respectively) and cytogenetic risk (adverse risk in 17% and 21%, respectively). Patients were followed for a total of 2090 person-years. Acute graft-versus-host disease grade II-IV occurred less frequently in NMA conditioned patients (20% [95% confidence interval (CI): 15%-26%] versus 38% [CI: 32%-45%] in MA conditioned patients, $p < 0.01$), while chronic graft-versus-host disease occurred in similar rates (50% [CI: 43%-56%] and 51% [CI: 44%-58%] in NMA and MA conditioned patients, respectively, $p = 0.77$). NMA conditioned patients had, however not with statistical significance, higher relapse rate (34% [CI: 28%-40%] versus 28% [CI: 22%-34%] in MA conditioned patients, $p = 0.07$) and lower NRM (20% [CI: 14%-25%] versus 25% [CI: 19%-31%] in MA conditioned patients, $p = 0.27$). Five-year overall survival was 55% (CI: 48%-62%) and 54% (CI: 47%-61%) in NMA and MA conditioned patients, respectively ($p = 0.9$).

Conclusions: Patients with AML undergoing allo-HSCT with NMA conditioning at our institution were older and frailer than MA conditioned patients, but their overall survival after transplantation was comparable. This might be explained by a generally lower AML stage and cytogenetic risk at transplant in NMA conditioned patients.

Lymphopenia and risk of infection and infection-related death in 98,344 individuals from a prospective Danish population-based study

Marie Warny, Jens Helby, Børge Nordestgaard, Henrik Birgens, and Stig Egil Bojesen

Department of Hematology, Department of Internal Medicine, and Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital

Background: Neutropenia increases the risk of infection, but it is unknown if this also applies to lymphopenia. We therefore tested the hypotheses that lymphopenia is associated with increased risk of infection and infection-related death in the general population.
Methods and findings: We analyzed 98,344 individuals from the Copenhagen General Population Study, with available blood lymphocyte count at date of examination. During a median of 6 years of follow-up, they developed 8,401 infections and experienced 1,045 infection-related deaths. Due to the completeness of the Danish civil and health registries, none of the 98,344 individuals were lost to follow-up. Individuals with lymphopenia (lymphocyte count < 1.1 × 10^9/l, n = 2,352) compared to those with lymphocytes in the reference range (1.1–3.7 × 10^9/l, n = 93,538) had multivariable-adjusted hazard ratios of 1.41 (95% CI 1.28–1.56) for any infection, 1.31 (1.14–1.52) for pneumonia, 1.44 (1.15–1.79) for skin infection, 1.26 (1.02–1.56) for urinary tract infection, 1.51 (1.21– 1.89) for sepsis, 1.38 (1.01–1.88) for diarrheal disease, 2.15 (1.16–3.98) for endocarditis, and 2.26 (1.21–4.24) for other infections. The corresponding hazard ratio for infection-related death was 1.70 (95% CI 1.37–2.10). Analyses were adjusted for age, sex, smoking status, cumulative smoking, alcohol intake, body mass index, plasma C-reactive protein, blood neutrophil count, recent infection, Charlson comorbidity index, autoimmune diseases, medication use, and immunodeficiency/hematologic disease. The findings were robust in all stratified analyses and also when including only events later than 2 years after first examination. However, due to the observational design, the study cannot address questions of causality, and our analyses might theoretically have been affected by residual confounding and reverse causation.
Conclusions: Lymphopenia was associated with increased risk of hospitalization with infection and increased risk of infection-related death in the general population.

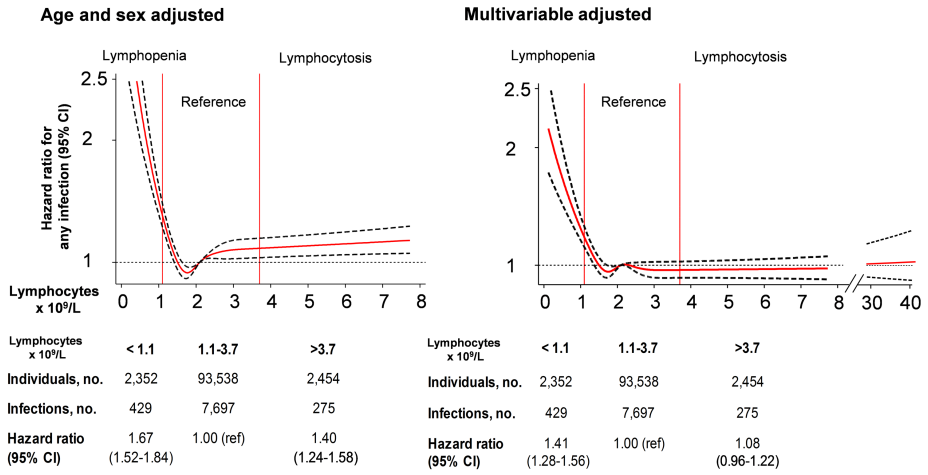


Figure: Risk of any infection as a function of lymphocyte count for individuals from the Copenhagen General Population Study

Peptide vaccination against PD-L1 (IO103) in multiple myeloma – a phase I first-in-human trial

Nicolai Grønne Jørgensen[1,4], Shamaila Munir Ahmad[1], Uffe Klausen[1,4], Jacob Handlos Grauslund[1], Mia Thorup Lundsager[1], Özcan Met[1,2,3], Inge Marie Svane[1,2], Lene Meldgaard Knudsen[4], Mads Hald Andersen[1]

[1]Center for Cancer Immune Therapy (CCIT), Department of Hematology, Herlev and Gentofte Hospital, Herlev, Denmark, [2]Department of Oncology, Herlev and Gentofte Hospital, Herlev, Denmark, [3]Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [4]Department of Hematology, Herlev and Gentofte Hospital, Herlev, Denmark

Background

The level of PD-L1 on multiple myeloma (MM) cells is associated with relapse, treatment refractory disease and an aggressive disease phenotype. Studies with therapeutic anti-PD-1 monoclonal antibodies in MM have so far produced discouraging results. We have developed a peptide vaccine against PD-L1. The vaccine consists of a 19-amino acid peptide from the signal peptide of PD-L1 (IO103), aqueously dissolved and emulsified with the adjuvant Montanide.

Aims

To assess safety of vaccination in patients with MM. Immunogenicity is a secondary outcome measure. Efficacy will be described, but the trial is not powered to assess efficacy due to the small sample size (NCT03042793).

Methods

Ten patients without severe co-morbidities or autoimmune diseases were recruited when they were between 4 weeks and 1 year from HDT. Patients were vaccinated subcutaneously with the peptide 15 times over the course of one year. Adverse events were assessed according to CTCAE v.4.01. Delayed type hypersensitivity (DTH) reaction against the vaccine was performed after 6 injections. 48 hours after injection, punch-biopsies were taken from the sites of DTH-injections. Skin infiltrating lymphocytes were grown from these biopsies, and their reactivity against the vaccine was tested in enzyme-linked immune-spot assays.

Results

Adverse reactions to the PD-L1 vaccine (IO103) have been mainly grade 1-2 injection site reactions. The rate of infections has been as expected for the population. Since the start of vaccinations in March 2017, 6 of 10 patients have experienced relapse of MM. 9 patients are still alive. The rate of relapses has been as expected for the population. 8 of 8 patients with evaluable skin-infiltrating lymphocytes show strong reactions to the vaccine (fig. 1).

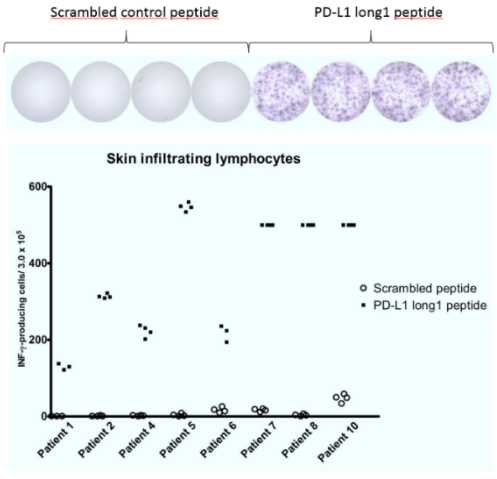


Fig 1. Reactivity of skin infiltrating lymphocytes.

Conclusion

Vaccinations with the PD-L1 peptide was associated with very limited side effects. The vaccine is highly immunogenic. A phase IIa study is ongoing in patients with high-risk smoldering multiple myeloma.

Immune electron microscopy and mass spectrometry for classification of amyloid deposits

Niels Abildgaard[1], Aleksandra Rojek[2], Hanne EH Møller[2], Nicolai Bjødstrup Palstrøm[3], Lars Melholt Rasmussen[3], Charlotte Toftmann Hansen[1], Hans Christian Beck[3] & Niels Marcussen[2]

[1]Hæmatologisk Afdeling; [2]Afdeling for Klinisk Patologi; [3]Klinisk Proteom Center, Afdeling for Klinisk Biokemi og Farmakologi; Odense Amyloidosis Center, Odense University Hospital.

Amyloidosis is a shared name for a number of rare, complex and serious disease entities with an annual incidence in Denmark of about 2-3 per 100,000 (2) which are caused by extra-cellular deposits of amyloid substance. The amyloid deposit is created by accumulation of different misfolded proteins. Accurate characterization of the amyloid protein is essential. Amyloid light chain (AL) amyloidosis is a plasma cell dyscrasia that can be treated with chemotherapy, whereas transthyretin (ATTR) and secondary Amyloid A (AA) amyloidosis are other entities that are not treated within hematology. It is generally recognized that immunohistochemistry is difficult to optimize for precise classification of amyloid. Internationally, immune electron microscopy (IEM) and mass spectrometry (MS) are the new gold standards. It has not been established if one of the methods is superior; each method have some strengths and weaknesses.

At Odense Amyloidosis Center, IEM and MS have been established and validated for standard diagnostics in 2017. As part of the validation we performed a retrospective study of 106 Congo-positive biopsies from a number of different involved organs; heart, kidney, lung, gut mucosa, subcutaneous fat, bone marrow. IEM was performed with gold-labeled antibodies against kappa, lambda, transthyretin and amyloid A. MS was performed on laser dissected amyloid protein obtained by laser-microscopy.

By MS a clear amyloid protein signature were identified in 102/106 biopsies (96.2%). In 6 biopsies the amyloid subtype could not be clearly identified but was clearly established in 96 biopsies (90.6%). By IEM 96/106 (90.6%) of the biopsies had amyloid fibrils and were positive by specific staining; the rest of the biopsies were either insufficient for examination or without amyloid fibrils, and in 1 biopsy the subtype of amyloid fibrils could not be established. The concordance of the results between the methods was 92.5%. The combined use of both methods increased the diagnostic sensitivity to almost 100%. Further analyses on the performance of the methods, and of potential differences in organ-specific sensitivity are ongoing but will be presented at the meeting along with an proposal for diagnostic algorithm.

Indications of improved primary hemostasis in patients with RR-CLL treated with ibrutinib and venetoclax.

Rebecka Svanberg, Sisse Rye Ostrowski, Carsten Utoft Niemann

Background: Recent clinical trial reports show that combined therapy with Btk-inhibitor ibrutinib and Bcl-2-inhibitor venetoclax is effective for patients with refractory/relapsed chronic lymphocytic leukemia (RR-CLL). While treatment-related bleeding constitutes a clinical challenge following ibrutinib monotherapy, it is uncertain to which degree bleeding occurs following combination therapy with ibrutinib and venetoclax, and it is not even mentioned among treatment-related adverse events for venetoclax. This study aimed to investigate the effect of both regimens on hemostasis in RR-CLL patients.

Methods: Peripheral blood samples from 7 patients enrolled in the VISION trial were taken at baseline, after 8 weeks of ibrutinib 420 mg daily as monotherapy, and after another 8 weeks of combined therapy with ibrutinib and venetoclax. We assessed primary hemostasis by Multiplate using three platelet activation stimuli (ADP, arachidonic acid (ASPI), and TRAP-6). Multiplate values were assessed as crude values and normalized to platelet counts. Secondary hemostasis was assessed by thromboelastography (TEG).

Results: Primary hemostasis was impaired in 6 out of 7 patients at baseline compared to normal reference levels, and it remained unchanged after 8 weeks of ibrutinib monotherapy apart from a slight improvement for ADP-stimulation. However, a distinct improvement in primary hemostasis was observed for all agonist stimuli following venetoclax addition. In contrast, TEG values were normal at baseline and remained unchanged throughout both treatments indicating no impairment of secondary hemostasis.

Conclusion: In this study, we show for the first time that primary hemostasis in RR-CLL patients improves significant following combination treatment with ibrutinib and venetoclax compared to baseline and ibrutinib monotherapy. Our findings indicate that bleeding risk associated with ibrutinib monotherapy can be reduced with venetoclax, which consequently could have valuable clinical implications. We are currently exploring whether our results are reflected in clinical observations by investigating bleeding events in the VISION cohort.

Prior clonal hematopoiesis and development of therapy-related AML/MDS in patients with lymphoma treated with high-dose chemotherapy

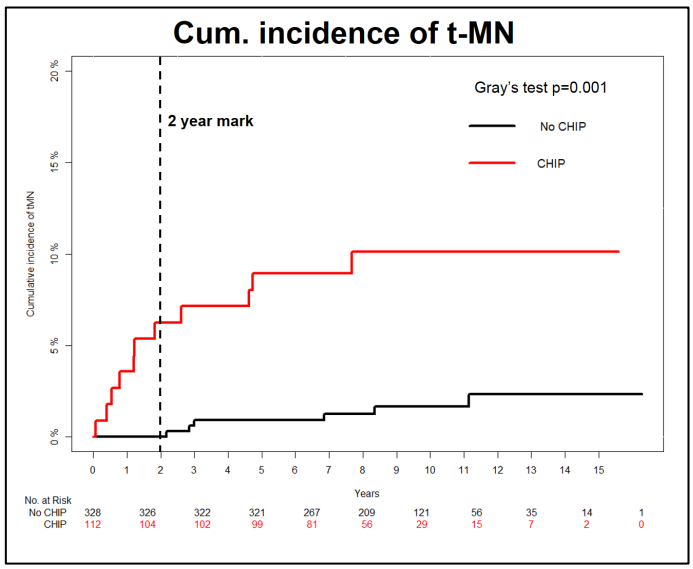
Simon Husby, Francesco Favero, Christian Nielsen, Betina Sørensen, John Bæch, Jakob W. Hansen, German G.R. Gonzalez, Eva K. Hastrup, Anne Fischer, Lisbeth Pernille Andersen, Bente Arboe, Susanne G. Sækmose, Per Boye Hansen, Ilse Christiansen, Erik Clasen-Linde, Lene Meldgaard, Kathrine Grell, Lene Hyldahl Ebbesen, Erik Kay Segel, Pär Josefsson, Michael Thorsgaard, Tarec C. El-Galaly, Peter Brown, Joachim Weischenfeldt, Thomas Stauffer Larsen, and Kirsten Grønbæk.

Therapy-related myeloid neoplasms (t-MN) are defined as leukemia or myelodysplastic syndromes that arise in patients (pts) after the administration of chemo- and/or radiotherapy. Outcomes after diagnosis of t-MN are dismal. Predicting pts at risk of developing t-MN is therefore of great importance. Here, we investigated clonal hematopoiesis of indeterminate potential (CHIP) in a national population-based cohort of lymphoma pts who were intended for high-dose chemotherapy (HDT) with stem cell support, and analyzed the effect on development of t-MN and overall survival.

All pts in Denmark with a lymphoma diagnosis, and a registered autologous stem cell sample at one of the six Danish transplant centers, between 2000 and 2012 were included. Next generation targeted deep sequencing (NGS) of CHIP genes was performed. Pts were followed via national Danish registries.

892 pts were identified; 440 pts fit inclusion criteria and had sufficient material for NGS. With a median follow-up of 9.1 years for surviving patients, a total of 17 pts (4%) developed t-MN. All patients who developed t-MN within two years after sampling (n=7) had identifiable CHIP mutations (Figure 1). Of the 440 pts, those with DNA repair pathway mutations (*PPM1D*, *TP53*, n=40) had significantly worse OS, and an increased risk of several adverse events, but had similar rates of t-MN as patients with other CHIP mutations (11% and 13%, respectively). However, when bone marrow pathology requisitions of patients with DNA repair mutations (n=40) were scrutinized, 8 pts were clinically suspected of t-MN (without morphologic dysplasia to confirm this), besides the 5 xpts diagnosed with t-MN.

In this population-based cohort of lymphoma pts, NGS analysis before administration of HDT identified CHIP in the majority of patients who developed t-MN. Additionally, we find that a third of patients with mutations in DNA repair pathway genes later were suspected of or diagnosed with t-MN.



DHS årsmøde

Title: **Immune recognition of neoantigens in myelodysplastic syndromes**

Author: **Staffan Holmberg[1,2]**

Co-authors: S. Kumar Saini[1], A.M. Bjerregaard[1], K. Grønbæk[3], A. Unnikrishnan[4], J. Pimanda[4], H. Medyouf[5], S. Reker Hadrup[1].

Affiliations: [1]Technical University of Denmark, Health Technology, Lyngby, Denmark.
[2]Herlev and Gentofte hospital, Dept of hematology, Herlev, Denmark.
[3]Rigshospitalet, Dept of hematology, Copenhagen, Denmark.
[4]Prince of Wales Clinical School, UNSW Medicine, Sydney, Australia.
[5]Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt, Germany.

ABSTRACT:

Immunotherapy is being thoroughly tested for hematological malignancies. In myelodysplastic syndromes (MDS), greatest focus has been towards vaccines and checkpoint inhibitors (CI).

The cancer types, where CI has shown the greatest clinical benefit, are the ones that carry a high mutational burden. Mutations create neoantigens, that can be presented on HLA molecules, and are suggested to be the main mediator of immunogenic cancer cell elimination. MDS carry comparably few mutations in their malignant cells. Therefore, it remains doubtful whether the malignant cells in MDS can be sufficiently immunogenic to generate an adequate immune response when treated with CI.

In this study we investigated T cell responses to personal neoantigens in patients with MDS. We ran DNA and RNA sequencing data, from mesenchymal stem cells and CD34+ cells in 15 patients with MDS, through an MHC binding prediction algorithm, to create personal libraries of neo-peptides corresponding to the individual patients' mutations. Bone marrow samples from the patients were screened for T cell binding to peptide-MHC-multimers (pMHC) coupled with a DNA-barcode, allowing us to look for T cell reactivity against more than 1000 pMHC in parallel. T cell responses were then validated using functional analysis.

Current results indicate that several neoepitopes in MDS are indeed immunogenic and are recognized by cytotoxic T cells in the bone marrow. Interestingly, many of the same neoepitopes are also recognized by T cells in healthy donors, but in contrary to the patient samples, neoepitope binding T cells from healthy donors are not functionally active.

To our knowledge, this is the first time neoantigens have been detected and functionally verified in the context of MDS. These findings could lead to further explorations into personal neoepitope vaccines, currently tested in other malignancies, and suggests a role for CI in MDS, possibly combined with specific neoepitope targeting.

Copy Number Variations Predict Poor Survival in Patients with Idiopathic Cytopenia of Undetermined Significance (ICUS) and are Associated with Macrocytosis

SU Mikkelsen,[1,2] S Safavi,[3] JW Hansen,[1,2,4] K Dimopoulos,[5] C O'Rourke,[2] MK Andersen,[3] JB Andersen,[2] and K Grønbæk[1,2,4]

[1]Department of Hematology, Rigshospitalet; [2]BRIC, University of Copenhagen; [3]Department of Clinical Genetics, Rigshospitalet; [4]DanStem, University of Copenhagen; [5]Department of Clinical Biochemistry, Rigshospitalet

Approximately half of ICUS patients have somatic mutations associated with increased risk of progression. The cause of cytopenia in the remaining ICUS patients is unknown. We hypothesize that uniparental disomy (UPD) and submicroscopic copy number variations (CNVs; gains and deletions) are present in ICUS patients and may be associated with prognosis.

Patients (n=154) referred with ICUS (2008-2017) were included if cytopenia persisted for >6 months, cytogenetics was normal and BM morphology was not diagnostic. SNP-A was performed on DNA from MNCs or granulocytes using Illumina Infinium-CytoSNP-850K and analyzed with GenomeStudio-v1.1 (Illumina). Only dels>30 markers, gains>90 markers and UPD>5Mb were reported.

A total of 33 CNVs/UPDs (excluding delY) were detected in 27/154 patients (18%). Mutations were present in 12/27 patients (44%) with CNVs/UPDs. Twenty-six deletions or UPDs (del/UPD) were detected in 21/154 patients (14%) of whom 11/21 (52%) harbored mutations.

After a median follow-up of 25 months (range, 2-114), median OS was 67 months (95%CI:19-not reached) in patients with del/UPD and not reached in patients without (p=0.003), *Fig.1*. The association with poor OS was also significant when including gains (p=0.02), however, the impact seemed driven by del/UPD, and here results with del/UPD are presented. In multivariate analysis, del/UPD (HR=2.7, 95%CI:1.22-5.9, p=0.014) and deep anemia (hgb<6.2) (HR=2.2, 95%CI:1.10-4.4, p=0.025) were independent adverse prognostic factors for OS, *Fig.2*. Interestingly, an almost identical pattern was observed between macrocytosis (MCV>100fL) and del/UPD indicating a linked impact on survival. Patients with del/UPD had significantly higher MCV (p=0.005) and P-ferritin (p=0.03). Importantly, macrocytosis was not associated with deep anemia (p=0.317).

To our knowledge, this is the first study investigating CNVs/UPDs in ICUS patients. Our results suggest that SNP-A can aid the prognostics in ICUS by identifying submicroscopic structural variations as risk markers for poor OS. A possible role of macrocytosis as a surrogate marker should be explored.

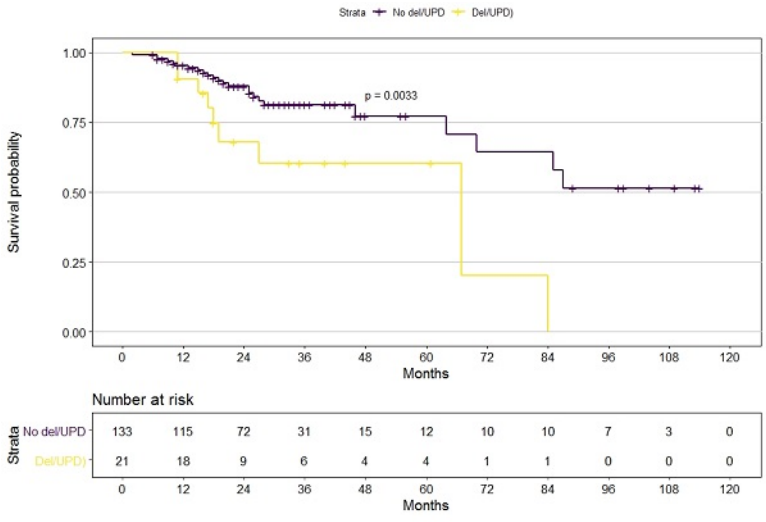


Figure 1. Kaplan-Meier survival curves for patients with deletions or UPD (del/UPD) and patients without del/UPD. Median overall survival (OS) was compared using a stratified log-rank test.

X axis: Time from first visit (study inclusion) in months.

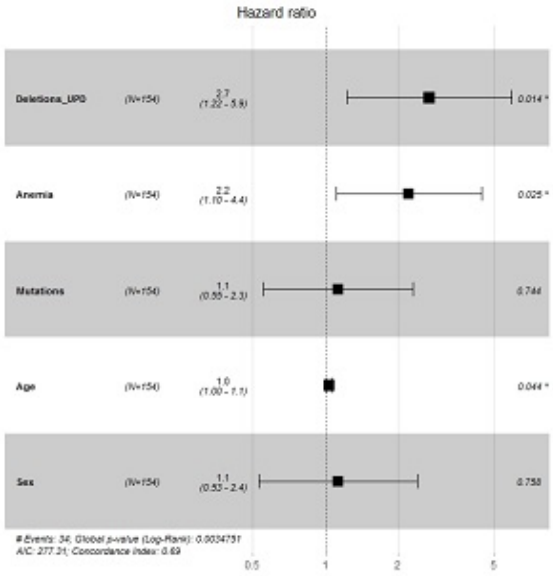


Figure 2. Forest plot with hazard ratios (HR). Deletions or UPD (Deletions_UPD) and macrocytosis were not included in the same model due to multicollinearity. Cox proportional hazard regression models were used to estimate HRs and associated 95% CIs. Anemia: hgb < 6.2 mM; Mutations: Somatic mutations with a VAF ≥ 5%.

Oral vitamin C supplementation to myeloid cancer patients on azacitidine treatment: Normalization of plasma vitamin C induces epigenetic changes

L.Gillberg,[1,2] A.D.Ørskov,[1,2] A.Nasif,[3] H.Ohtani,[4] Z.Madaj,[4] J.W.Hansen,[1,2,7] N.Rapin,[2] J.B. Mogensen,[1,2] M.Liu,[4] I.H.Dufva,[5] J.Lykkesfeldt,[6] P.Hajkova,[3] P.A.Jones,[4] K.Grønbæk[1,2,7]

[1]Dept. of Haematology, Rigshospitalet. [2]BRIC, University of Copenhagen; [3]MRC London Institute of Medical Sciences (LMS), Imperial College, London, UK; [4]Van Andel Research Institute, Grand Rapids, Michigan, USA; [5]Department of Haematology, Herlev University Hospital; [6]Department of Veterinary and Animal Sciences, University of Copenhagen; and [7]The Danish Stem Cell Center (Danstem), University of Copenhagen, Denmark.

Abstract

Purpose: Hematological cancer patients are often vitamin C deficient, and vitamin C is essential for the TET-induced conversion of 5-methylcytosine (5mC) to 5-hydroxy-methylcytosine (5hmC); the first step in active DNA demethylation. Here, we investigated whether oral vitamin C supplementation can correct vitamin C deficiency, enhance the 5hmC/5mC ratio and upregulate expression of viral defense genes in myeloid cancer patients treated with DNA methyltransferase inhibitors (DNMTis).

Experimental design: A randomized, placebo-controlled clinical trial in myeloid cancer patients performed during 3 cycles of DNMTi-treatment (5-azacitidine, 100 mg/m^2/d for 5 days in 28-day cycles) supplemented by oral dose of 500 mg vitamin C (n=10) or placebo (n=10) daily during the last 2 cycles (Figure 1).

Results: Fourteen patients were deficient in plasma vitamin C (<23 μM) and four of the remaining six patients were taking vitamin supplement at inclusion. Global DNA methylation was significantly higher in patients with severe vitamin C deficiency (<11.4 μM; *P*=0.004). Oral supplementation restored plasma vitamin C levels to the normal range in all patients in the vitamin C arm (P=0.0004). We show for the first time that global 5hmC/5mC levels were significantly increased in patients receiving vitamin C compared to placebo (*P*=0.041). Additionally, preliminary data suggest that vitamin C supplement may increase the upregulation of viral defense genes in DNMTi naïve patients.

Conclusions: Normalization of plasma vitamin C by oral supplementation may enhance the biological effects of DNMTis in patients and prompts the investigation of the clinical efficacy of vitamin C supplementation to DNMTis in a large randomized, placebo-controlled trial.

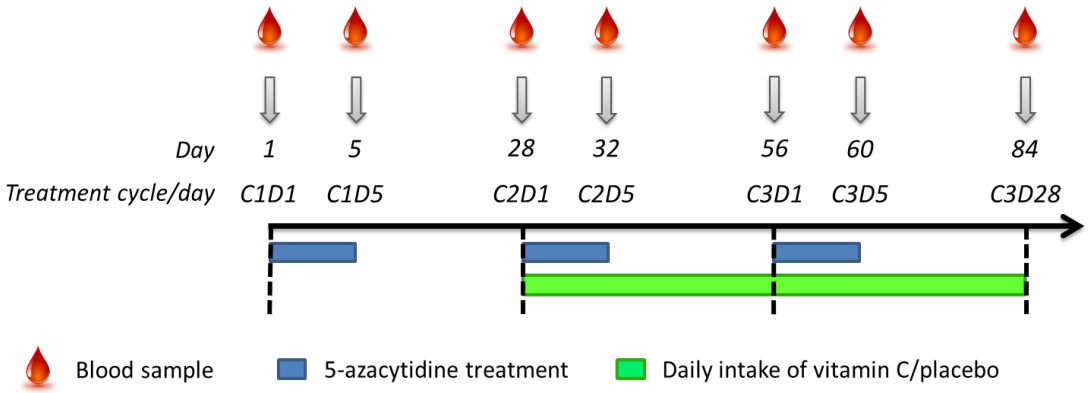


Figure 1. Study design. Days 1, 5, and 28: before 500 mg vitamin C/placebo exposure. Day 32: after short-term vitamin C/placebo exposure. Days 56, 60, and 84: after longer-term vitamin C/placebo exposure.

DEPRESSION AND ANXIETY IN HODGKIN LYMPHOMA SURVIVORS: A DANISH NATIONWIDE COHORT STUDY OF 896 PATIENTS

Andreas Kiesbye Øvlisen[1,2], Lasse Hjort Jakobsen[1,2], Kristian Hay Kragholm[3], Martin Hutchings[4], Christian Bjørn Poulsen[5], Henrik Frederiksen[6], Danny Stoltenberg[7], Martin Bøgsted[1,2], Lene Sofie Granfelt Østgård[8], Marianne Tang Severinsen[1,2,3], Tarec Christoffer El-Galaly[1,2,3].

1. Department of Haematology, Aalborg University Hospital, Aalborg, Denmark
2. Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
3. Unit of Epidemiology and Biostatistics, Aalborg University, Aalborg, Denmark; Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark
4. Department of Haematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
5. Department of Haematology, Zealand University Hospital, Roskilde, Denmark
6. Department of Haematology, Odense University Hospital, Odense, Denmark
7. Department of Haematology, Copenhagen University Hospital, Herlev, Denmark
8. Department of Haematology, Aarhus University Hospital, Aarhus, Denmark
9. Clinical Cancer Research Unit, Aalborg University Hospital, Aalborg, Denmark

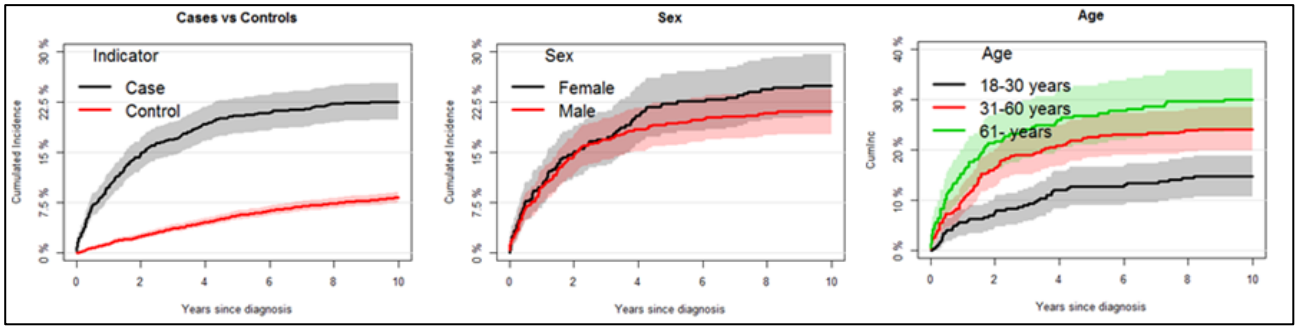
Cancer related psychological distress can lead to depression and anxiety among survivors. Despite the vast majority of patients with Hodgkin Lymphoma (HL) become long-term survivors, the risk of mental health problems is not well described. We aimed to compare the use of psychotropic drugs (PD) (antidepressants, anxiolytics, and antipsychotics) in HL patients to a matched background population. HL patients diagnosed in the period 2005-2015 were retrieved from LYFO and merged with the Danish National Prescription Registry. For each HL patient, five random persons from the Danish population were matched on month of birth and gender. All was alive at the date of diagnosis of the index patient and without PD prescriptions before that date.

896 HL patients were included (male:female ratio 1.7, median age 39 years, follow-up 7.5 years). 204 HL patients (22.8%) received PD after diagnosis compared to 384 persons (5.6%) in the matched cohort. HL patients were more likely to receive PD (HR 3.4, 95% CI 2.8-4.0) compared to the background population (Figure 1). Age, ECOG and stage were associated with PD prescriptions (Table 1). The relative risk of PD prescription was highest for HL patients at diagnosis but gradually normalized for patients who did not receive PD in the first years. (RR 8.3, P<0.01 within the first year; RR 5.2, P<0.01 within the second year; RR 1.1, P=0.6 between two and five years).

Table 1: Impact of various variables on PD use for HL patients

Variables	HR	95% CI	p-value
Baseline characteristics			
Sex			
Male	1.00	(reference)	
Female	1.17	0.89-1.55	0.264
Age			
18-30 years	1.00	(reference)	
31-60 years	1.64	1.15-2.34	0.006
61- years	2.86	1.95-4.19	<0.001
ECOG			
0	1.00	(reference)	
1	2.02	1.49-2.73	<0.001
≥2	2.80	1.63-4.79	<0.001
Ann Arbor Stage			
Stage I-II	1.00	(reference)	
Stage III-IV	1.75	1.33-2.31	<0.001
Charlson Comorbidity Index			
0	1.00	(reference)	
1	2.25	1.19-4.26	0.013
≥2	1.08	0.48-2.43	0.854

HL patients have increased risk of depression and anxiety symptoms reflected by the use of PD. However, few years into survivorship PD prescription rate normalized to that of the background population. Young HL patients (≤30 years) had lower incidence of PD prescriptions as compared to elderly (≥31 years). In conclusion, screening for symptoms of depression and anxiety is warranted in the time after diagnosis and first years into survivorship.



Figur 1: Cumulative incidences of receiving prescriptions for PD illustrated with 95% confidence intervals.

IGHV mutational status and outcome for patients with chronic lymphocytic leukemia upon treatment: a nationwide population-based study

E. C. Rotbain[1,2], H. Frederiksen[1,3], H. Hjalgrim[2,4], K. Rostgaard[4], G. J. Egholm[1], B. Zahedi[2], C. B. Poulsen[5], L. Enggard[6], C. da Cunha-Bang[α2], C. U. Niemann[α2]

[1] Department of Hematology, Odense University Hospital, Denmark, [2] Department of Hematology, Rigshospitalet, Denmark, [3] Academy of Geriatric Cancer Research (AgeCare), Odense University Hospital, [4] Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark, [5] Department of Hematology, Zealand University Hospital, Denmark, [6] Department of Hematology, Herlev Hospital, Denmark, [α]Co-senior authors

Background: Patients with chronic lymphocytic leukemia (CLL) who have immunoglobulin heavy-chain variable region gene unmutated status (IGHV-U) have inferior survival from time of treatment in clinical studies, while real-world data are lacking.

Methods: This population-based cohort comprises all 4152 patients from the Danish CLL-register, diagnosed between 2008-2017. For half of the population, detailed data was collected from patient records, for all patient who had received treatment (n=502), to evaluate treatment-free survival from first line treatment (TFS).

Results: IGHV-status was known for 77%, of which 32% were IGHV-U. In total, 857 patients with known IGHV-status received treatment: 42% patients received intensive chemoimmunotherapy (CIT) treatment in terms of fludarabine, cyclophosphamide and rituximab (FCR) or bendamustine and rituximab (BR), 26% chlorambucil in combination with anti-CD20 antibodies (CD20Chlor) or as monotherapy (Chlor) and 32% received other, less common, treatments. Overall survival (OS) from time of treatment was independent of IGHV-status for all treatment regimens. TFS from time of first line treatment was statistically significantly inferior for IGHV-U patients, compared with IGHV-M patients, when treated with FCR, BR or non-intensive treatment regimens, HR 2.56, 7.50 and 2.04, respectively (Fig. 1). The median TFS was 2.5 and 1 year for CD20Chlor- and Chlor-treated patients, respectively. The 3-year TFS for FCR- and BR-treated patients, was 90% and 91% for IGHV-M and 76% and 53% for IGHV-U, respectively.

Conclusion: Intensive CIT provides a long treatment-free survival for IGHV-M patients and a possible cure with FCR. FCR is a valid treatment option for IGHV-U patients also, where a lengthy TFS may be achieved. Better supportive care or improved treatment options are warranted for IGHV-U patients who are unfit for FCR-treatment and for all patients unfit for intensive CIT.

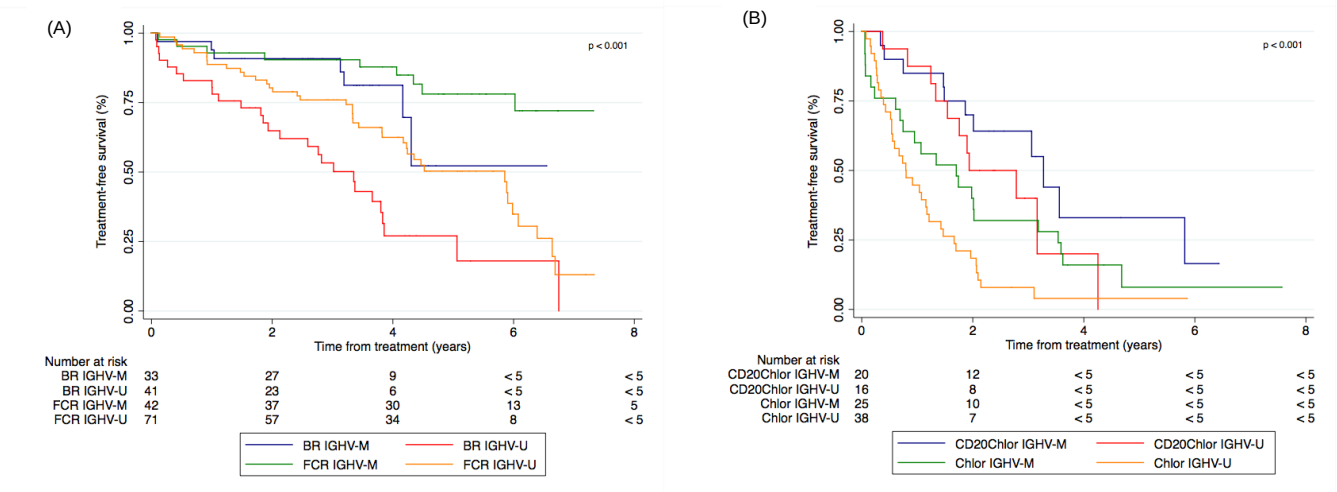


Fig 1. Treatment-free survival from start of first line treatment for immunoglobulin heavy-chain variable region gene mutational status mutated (IGHV-M) and unmutated (IGHV-U) patients treated with either fludarabine, cyclophosphamide and rituximab (FCR) or bendamustine and rituximab (BR) (A) or Chlorambucil (Chlor) or Chlorambucil and anti-CD20 CD20Chlor (B).

Strategier til forbedring af patient-reported outcome data komplethed

Lene Kongsgaard Nielsen[1,2], Sören Möller[2,3], Mary Jarden[4], Christen Lykkegaard Andersen[4], Henrik Frederiksen[1], Henrik Gregersen[5], Anja Klostergaard[6], Morten Saaby Steffensen[7], Per Trøllund Pedersen[8], Maja Hinge[9], Mikael Frederiksen[10], Bo Amdi Jensen[11], Carsten Helleberg[12], Anne Kærsgaard Mylin[4], og Niels Abildgaard[1,13]

[1]Quality of Life Research Center, Hæmatologisk afdeling, Odense Universitetshospital; [2]OPEN, Odense Patient data Explorative Network, Odense Universitetshospital; [3]Klinisk Institut, Syddansk Universitet; [4]Hæmatologisk Klinik, Rigshospitalet; [5]Hæmatologisk afdeling, Aalborg Universitetshospital; [6]Hæmatologisk afdeling, Aarhus Universitetshospital; [7]Medicinsk afdeling, Regionshospitalet Vest; [8]Hæmatologisk afdeling, Sydvestjysk Sygehus; [9]Hæmatologisk afdeling, Vejle Hospital; [10]Hæmatologisk dagklinik, Sygehus Sønderjylland; [11]Hæmatologisk afdeling, Sjællands Universitetshospital, Roskilde; [12]Hæmatologisk afdeling, Herlev Hospital; [13]The Academy of Geriatric Cancer Research (AgeCare), Odense Universitetshospital.

Introduktion: Patient-reported outcomes (PRO) anvendes i tiltagende grad som endepunkt i kliniske studier til at vurdere patientoplevede fordele og ulemper ved behandlingen. PRO-resultater kan være behæftet med begrænsninger, hvis der er ikke-besvarede spørgeskemaer (non-responses, NR) i datasættet, særligt, hvis årsagen til NR er relateret til patientens helbred. PRO-data kompletheden i longitudinelle studier med patienter med myelomatose (MM) er fundet til 78-98 %, men denne procentdelen og årsagerne til NR publiceres ofte ikke. Formålet med denne analyse er at undersøge effekten af strategier til forbedring af PRO-data komplethed i et aktuelt studie af danske patienter med MM.

Metode: "Livskvalitet hos danske patienter med myelomatose" er et dansk multi-center studie med inklusion af behandlingskrævende patienter med nydiagnostisteret eller relaps MM. Deltagerne besvarer spørgeskemaer 13 gange over 2 år på papir eller elektronisk via e-mail. De involverede sygeplejersker bliver undervist i vigtigheden af reduktion af NR. Patienterne har et tidsvindue på 7 døgn til at besvare spørgeskemaet og ved NR udsendes påmindelse dag 4 til dem, som besvarer elektronisk. Ved fortsat NR på dag 7 sendes en notifikation til sygeplejersken, som kontakter patienten, afdækker årsagen til NR og opfordrer patienten til at afgive besvarelsen. Effekten af strategierne undersøges ved at udregne PRO-data komplethed indenfor 7 døgn-vinduet og i alt.

Resultater: Analysen omfatter de første 271 inkluderede patienter, hvoraf 249 patienter (85%) havde valgt elektronisk besvarelse. I alt 1441 spørgeskemaer var planlagte på analysetidspunktet og 1214 (84%) var besvaret indenfor 7 døgn og yderligere 153 (11%) blev besvaret efter kontakt til patienterne. Dette giver en samlet PRO-data komplethed på 95%.

Konklusion: De anvendte strategier havde god effekt på PRO-data komplethed og anbefales anvendt i kommende longitudinelle PRO-studier. Det skal bemærkes, at der er behov for personaleressourcer til opgaven. Årsagerne til NR bliver dokumenteret som en del af studiet og vil indgå i kommende sensitivitetsanalyser.

Preliminary safety profile of IO103 (PD-L1) and IO120 (PD-L2) peptide vaccine in relapsed follicular lymphoma

Uffe Klausen[1], Shamaila Ahmad[1], Nicolai Grønne Dahlager Jørgensen[1], Jacob Handlos Grauslund[1,2], Morten Orebo Holmström[1,2], Per Boye Hansen[2], Inge Marie Svane[1,3], Lars Møller Pedersen[1], Mads Hald Andersen[1,4]

[1] Center for Cancer Immune Therapy, department of hematology, Copenhagen university hospital Herlev
[2] Department of hematology, Zealand University Hospital
[3] Department of Oncology, Copenhagen university hospital Herlev
[4] Department of Immunology and Microbiology, University of Copenhagen, Copenhagen Denmark

Background: Follicular lymphoma (FL) is mostly an indolent but incurable disease and current treatments are associated with severe side effects such as neutropenia. The tumor microenvironment is important to growth and persistence of the lymphoma, and checkpoint molecules such as programmed death ligands 1 and 2 (PD-L1 and PD-L2), which inhibit T-cells from effectively clearing the malignant cells. These ligands are expressed on lymphoma cells and on tumor-associated macrophages. If safe, vaccination with PD-L1 and PD-L2 peptides might be able to raise an immune response towards lymphoma cells and supportive cells in FL patients.

Methods: A first-in-human phase I study evaluating the safety by CTCAE v4.03 of IO103 and IO120, PD-L1 and PD-L2 peptides in ten FL patients. Dose limiting toxicities was defined as three or more grade III+ adverse reactions. The vaccine is offered to patients with at least partial remission to standard chemotherapy. 15 vaccines will be administered over the course of one year.

Results: As of January 2019, 7 out of 10 patients have been included. Five patients have reached the interim time point of six vaccines with a mean of 9 (6-13). The minimum number of prior anti-cancer therapies is 3 (3-4) and mean time since diagnosis is 7,6 years (3-12). Two patients have been excluded due to progression during vaccination. No DLT was observed. Three patients experienced neutropenia related to previous chemotherapy and progression. Only CTCAE grade 1 adverse events have been related to the vaccine with injection site reactions being the most common event. Diarrhea and fatigue grade 1 was reported as related to the vaccine (table 1).

Conclusion: In this interim data set, the vaccine seems safe with only grade one adverse events related to the vaccine.

Table 1.

Adverse events (AE) with possible relation to the vaccine			
	Grade	Number of events	Number of patients
Injection site reaction	1	5	2
Diarrhea	1	3	1
Fatigue	1	3	2
Bursitis	1	1	1
Myalgia	1	1	1
Adverse Events grade 3+ and Serious Adverse Events not related to the vaccine			
(AE) Neutropenia	4	3	3
(SAE) Admission due to atrial fibrillation	3	1	1

Epidemiology outcomes and risk factors associated with Richter's transformation in patients with CLL

Authors:

Mariam Hussein Hleuhel (MH)*[1], Yasmin Ben-Dali (YB)*[1], Caspar da Cunha-Bang [1,2], Christian Brieghel[1], Christian Poulsen[3], Erik Clasen-Linde[4], Hans Herluf Nørregaard Bentzen[5], Henrik Frederiksen[6], Ilse Christiansen[7], Linda H. Nielsen[8], Lisbeth Enggaard[9], Marie Helleberg[10], Michael Roost Clausen[11], Mikael Frederiksen[12], Robert Schou Pedersen[13], Carsten Utoft Niemann[+1], Michael Asger Andersen[+1]

Background:

Richter's transformation (RT) refers to the development of an aggressive lymphoma in patients with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL). Approximately 2-10% of patients with CLL develop RT, most often as diffuse large B-cell lymphoma (DLBCL) or Hodgkin lymphoma (HL).

Aim:

The aim of this study was to assess the incidence of RT, risk factors for RT and survival among patients with RT in a nationwide CLL/SLL cohort.

Methods:

All patients diagnosed with CLL/SLL in Denmark between 2008 and 2016 were included. Data was retrieved from the Danish National CLL Registry and the Danish National Pathology Data Bank. Follow-up ended at date of diagnosis of RT, death or end of 2016, whichever came first. Cox models were used to evaluate hazard ratios of different risk factors for RT.

Results:

Among 3772 CLL patients, 113 patients had a biopsy-proven RT diagnosis after a median follow-up of 4.3 years. The 5-year cumulative incidence of RT was 2.8% (Figure 1). In a multivariable analysis, advanced Binet stage (B or C) (hazard ratio [HR], 2.8, P <0.001), unmutated IGHV status (HR, 2.3, P <0.001) and del(17p) (HR, 3.9, P <0.001) were independently associated with risk of RT.

Half of the patients with RT (49%) had never received any CLL-specific treatment prior to transformation and patients previously treated for CLL had a worse median overall survival (OS) (2.8 years) compared to treatment naïve RT patients (6.1 years) (Figure 2).

Conclusion:

We found a 5-year cumulative incidence of 2.8% among unselected patients with CLL. Approximately half of the patients with RT never received any CLL-specific treatment. The prognosis of RT was surprisingly good compared to previous reports especially for treatment-naïve patients.

Figures:

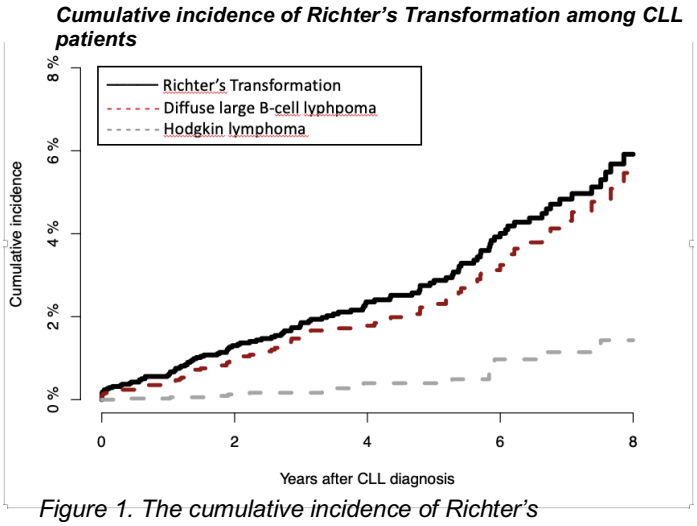


Figure 1. The cumulative incidence of Richter's Transformation in CLL patients from time of CLL diagnosis

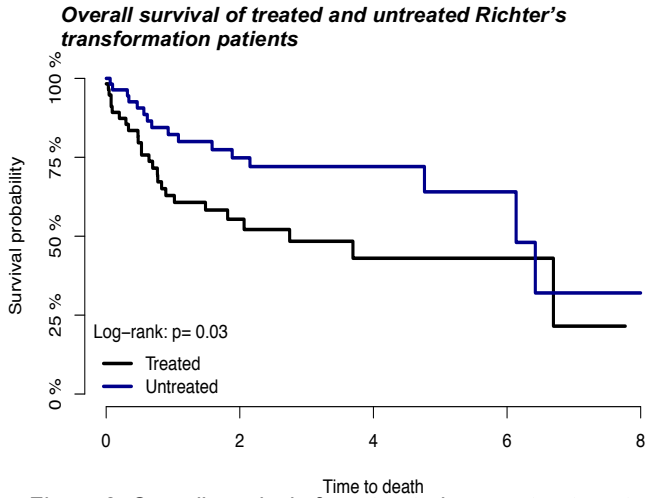


Figure 2. Overall survival of pre-treated versus treatment naïve Richter's Transformation patients

Author affiliations

* Co-authorship, MH and YB contributed equally to this paper, +co-senior authors
[1] Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Denmark
[2] Department of Hematology, Roskilde University, Denmark
[3] Department of Hematology, Roskilde University, Denmark, Herlev Hospital, Denmark
[4] Department of Pathology, Rigshospitalet, Copenhagen University Hospital, Denmark
[5] Department of Hematology, Aarhus University Hospital, Denmark
[6] Department of Hematology, Odense University Hospital, Denmark
[7] Department of Hematology, Aalborg University Hospital, Denmark
[8] Department of Hematology, Sydvestjysk Sygehus, Denmark
[9] Department of Hematology, Herlev and Gentofte Hospital, Denmark
[10] Department of Infectious Diseases, Rigshospitalet, Copenhagen University Hospital, Denmark
[11] Department of Hematology, Sygehus Lillebaelt, Denmark
[12] Department of Hematology, Sygehus Soenderjylland, Denmark
[13] Department of Hematology, Hospitalsenheden Vest, Denmark

The *KIT*D816V mutation is a target of specific T cells

Morten Orebo Holmström[1,2], Stine Emilie Weis-Banke[2], Shamaila Munir Ahmad[2], Hans Hasselbalch[1], Mads Hald Andersen[2,3]

[1] Department of Hematology, Zealand University Hospital
[2] Center for Cancer Immune Therapy, Herlev Hospital.
[3] Department of Immunology and Microbiology, University of Copenhagen

Background: The *KIT*D816V is a somatic mutation identified in a subgroup of patients with myeloid malignancies. The mutation generates a neo-antigen, that could possibly be recognized by specific T cells.

Aim: Clarify if the *KIT*D816V mutation is recognized by specific T cells

Methods: T cell responses from healthy donors were evaluated using Enzyme Linked Immuno Spot (ELISPOT), intracellular cytokine staining (ICS) and chromium-51 cytotoxicity assay.

Results: By using interferon-gamma ELISPOT we analyzed 35 healthy donors for spontaneous immune response against the KITLong epitope, which is a 29-mer epitope spanning the D816V amino acid substitution. Of the 35 healthy donors analyzed, 24 (69%) displayed a statistically significant response **(A).** By analyzing the KITLong specific responses with ICS, the responses were identified to be $CD4^+$ T cell responses **(B)**. A $CD4^+$ KITLong specific T cell culture from a healthy donor was established by repeated stimulations with KITLong pulsed autologous dendritic cells. KITLong specific $CD4^+$ T cell clones with no cross reactivity to the KITLong wild type epitope **(C)** was established by limiting dilution of sorted cells. The KITLong specific T cells were able to kill autologous dendritic cells pulsed with KITLong, however almost no killing was observed against autologous dendritic cells pulsed with KITLong wild type epitope **(D)**. The killing of the KITLong pulsed autologous dendritic cells was likely mediated by granzyme-perforin mediated cytolysis, as we detected an upregulation of the degranulation marker CD107a on T cells stimulated with dendritic cells pulsed with KITLong **(E)**.

Conclusion: The *KIT*D816V mutation is recognized by specific T cells. The potential of therapeutic cancer vaccination with *KIT*D816V derived epitopes in *KIT*D816V-mutant hematological cancer is relevant to investigate. The T cell responses in healthy donors suggest that healthy donors eliminate KITD816V mutant cells before development of malignancy, but this is yet to be investigated.