62nd ASH Annual Meeting and Exposition

Thanks to MDS Foundation and MDS Alliance I have been attending the 62nd ASH Annual Meeting and Exposition as a patient advocate associated with LyLe - the support group for lymphoma, leukemia and MDS in Denmark. This was an all virtual event, so I participated through the internet from Slangerup, Denmark.

Caveat: The viewpoint in this document is that of a patient, and not a doctor. Keep that in mind, as you read it. Also this report is written for patient advocates, who have gone through the WECAN Masterclass or have equivalent knowledge.

A virtual event has some advantages and some drawbacks. One of the advantages was that you can watch many pre-recorded presentations at any time, e.g. presentations from educational sessions or presentations from Fridays Satellite Symposia. You can even watch one event as it happens, and another parallel event later in the day or even next morning. Another advantage was that posters had been replaced with 4-5 minute pre-recorded presentations of each poster available throughout the duration of the meeting, and not just one day or one evening after a busy day running from session to session. Also in the poster area one could easily search for the ones of interest, e.g. those related to MDS.

A major drawback of the virtual event is the missing servings of good coffee and cookies in the exhibition area, which I enjoyed tremendously at the ASH Annual Meeting I attended years ago in San Diego. Luckily Philips Senseo makes excellent single cups of good coffee. However, I did not miss the huge amount of paper I returned from San Diego with.

Biggest MDS take away

My biggest MDS related take away from this year's virtual ASH Annual Meeting was from Saturday's educational session on MDS where, Dr. Bart Scott said that for higher risk MDS patients participation in a clinical trial should be considered first line treatment.

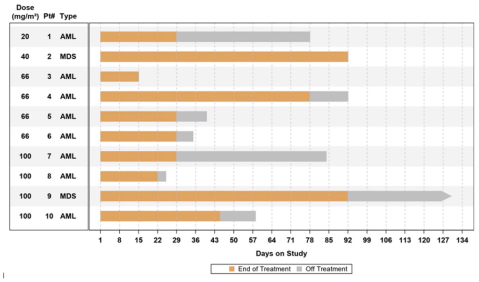
Biggest overall take away

The biggest overall meeting take away was a new treatment for beta-thalassemia and sickle cell diseases using CRISPR-CAS9, which showed these very transfusion dependent patients became transfusion independent after the treatment. Crisper is a technology for editing genes. At the time for data cut-off for ASH, only 10 persons had been treated - 7 with beta-thalassemia and 3 with sickle cell disease. All were transfusion independent a year after treatment. I would expect to see more Crisper based treatment in the coming years.

MDS Posters at 62nd ASH Annual Meeting

I view the most interesting posters to be those about APTO-252, Nivolumab and Ipilimumab with or without AZA, and sabatolimab all aimed at treating higher risk MDS patients as well as the study attempting to extend the use of Luspatercept to a larger group of low risk MDS patients. (click on links to jump directly to description of those posters). This is one of several new AZA combination treatments currently being investigated in chemical trials.

APTO-253 is a small molecule organic fluoride, which reduces MYC mRNA rather quickly - see figure 1. MYC is one of the most amplified oncogene in many different human cancers, including AML and MDS. Results on APTO-252 in humans are very preliminary, and the phase 1a/b study is ongoing and doesn't include PROM. MDS patients were longest on study. (abstract 1042)



Using double immune checkpoint inhibitor blockade with nivolumab and ipilimumab with azacitidine showed 67% CR in frontline treatment of higher-risk MDS, but only 36% CR in

treatment after HMA failure. A randomized clinical trial is needed to further investigate this treatment. (abstract 2203)

Gene sequencing may have a great potential in diagnosis of many hematological diseases, but what is gained by whole-genome sequencing (WGS) compared to just a panel of 12 genes as recommended by National Comprehensive Cancer Network (NCCN)? Researchers at the Munich Leukemia Laboratory conclude that from a clinical point of view almost nothing is gained by WGS. Personally I think that there is a need to further understand what one should be looking for in a WGS of MDS patients. (abstract 2180)
An Italian / British study found that clinically relevant features and endpoints could be identified in lower-risk MDS using genomic profiling. (abstract 2179)
Furthermore the translational research program at Rigshospitalet has decided to use WGS on all AML and MDS patient samples from patients enrolled in the program. And at EHA 2020 a schwiss researcher also recommended doing WGS of older non-cryo preserved samples to understand what worked and did not work in patients treated in the past.

The EBMT Chronic Malignancies Working Party (CMWP) asked if a change in IPSS-R between diagnosis and transplant effects transplant outcome. In my view that is a rather dum question to ask, since both the IPSS and the IPSS-R have been developed to estimate prognosis at time of diagnosis, and hence should not have power to estimate at time of transplant - especially considering the many factors that influence the time between diagnosis and transplant in different MDS patients. Also the EMBT CMWP don't actually calculate the change in IPSS-R for individual patient's and plot that change as a function of Overall Survival (OS) and Progression Free Survival (PFS). (abstract 2438)

Dr. W. Jiang report on infusion of 7 patients (4 AML and 3 higher-risk MDS) with donor-derived tumour associated antigen (TAA) specific and multipathogen (MP) specific T cells administered prophylactically to prevent relapse and infections after Hematopoietic Stem Cell Transplantation (HSCT) with 4 patients on ongoing followup for up to +900 days post transplant. Two of the other patients died, and one developed chronic GVHD. (abstract 2338)

Dr. J. Teichman asked if transferrin saturation correlates with ferritin levels, since ferritin is an imperfect measure of iron overload, as some iron is non-transferrin bound or labile plasma iron, that is not easily measured. The answer is NO, as seen on this scatter plot to the right the correlation is weak with an R-value of just 0.63. However, a relationship between transfusion burden and survival of lower risk MDS patients was found with no transfusions and low transfusion burden showing significant survival benefits. (abstract 2197)

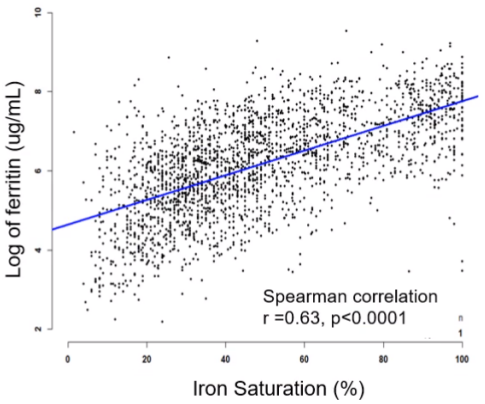


Table 1 Poster I looked at during ASH 2020 Virtual. The abstract # contains link to abstracts, and I have copies of most presentation slides.

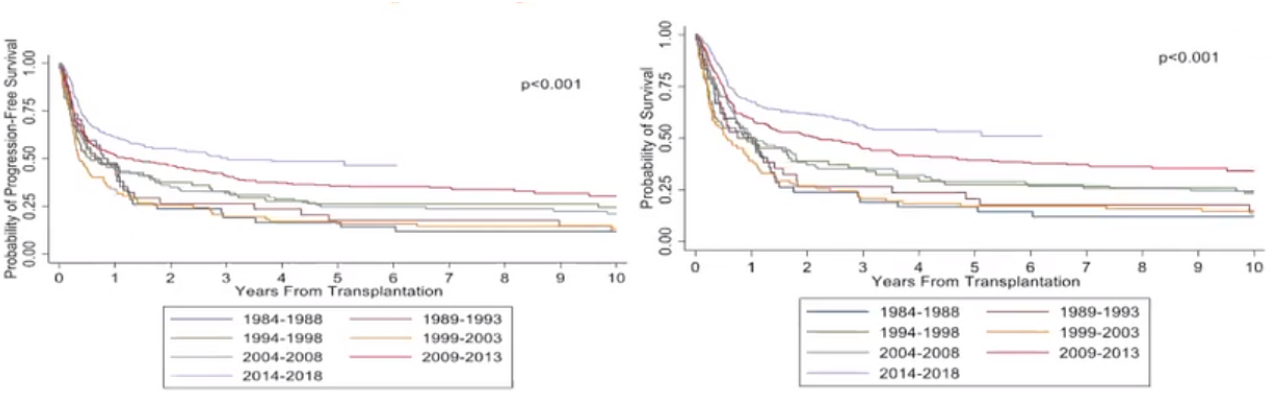
Abstract	Phase	Type	Disease	Remarks
1042	1a/b	Dose escalation study of the MYC repressor APTO-253	Relapsed or refractory AML or higher-risk MDS	No PROM, study ongoing and no OS or PFS data.

2203	2	Double immune checkpoint inhibitor blockade with Nivolumab and Ipilimumab with or without azacitidine	Higher-risk MDS frontline or after Hypomethylating Agents (HMA) failure	CR 67% in frontline and 37% after HMA failure. Need a randomized trial.
2180		WGS vs 12 gene panel recommended by NCCN	MDS	More learning needed to take advantage of WGS.
2179			Lower-risk MDS	
2438	Retro	Change in IPSS-R between diagnosis and transplant	MDS	Change in IPPS-R calculated at time of diagnosis to time of transplant has no influence on outcome.
2338	1	Donor-derived t-cells specific for WT1 and PRAME in combination with T-cells specific for multiple pathogens for prevention of relapse and infection after HSCT	AML and high-risk MDS	7 of 20 patients enrolled, 2 died and one has chronic Graft versus Host Disease (GVHD). Ongoing trial.
2197	Retro	Iron saturation versus ferritin as measure of iron overload	low risk MDS	Weak correlation between ferritin and iron saturation.
1905	2	Genetic abnormalities and Minimal residual disease (MRD) negativity	high risk MDS and AML	No impact of genetic abnormalities on MRD negativity.
2427	Retro	Survival of AML and MDS patients after HSCT	AML and high risk MDS	Significant improvement since 2004.
2403	Retro	Predict relapse from blood samples using PCR for patient specific biomarkers	MDS patients after HSCT	Power to predict established. Study of prediction starts in 2021.
2193	Retro	Red blood cell transfusion burden in MDS-RS	MDS-RS	Demonstrate the need of MDS-RS for luspatercept.
2192	1	Safe dose and effect of sabatolimab	AML and high risk MDS	Safe dose established for STIMULUS trial.
2374	Retro	Epstein-Barr-Virus (EBV) infection after T-cell	AML and high risk MDS	Nothing about how to cope with this.

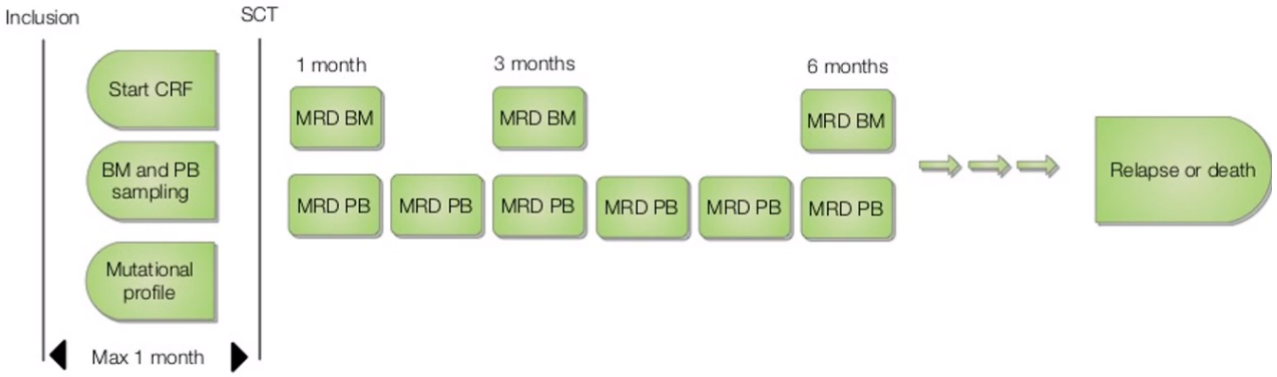
		depletion and HSCT		
2198	3	Luspatercept vs epoetin alfa in treatment naive MDS	Very low, low or intermediate MDS after IPSS-R	Recruiting, but not in Denmark.
1948	2	PFS and OS with reduced dose Azacitidine (AZA) for 5 days SC each monthly cycle	AML after MDS or higher risk MDS	This was an observational study only.

Dr. S. Kayser presented a poster on the impact of genetic abnormalities and MRD levels on outcome in patients with higher risk MDS or AML pre-emptively treated with azacitidine during the RELAZA2 trial. She found no impact of genetic abnormalities on MRD negativity as measured by quantitative Polymerase Chain Reaction (qPCR). However, neither this or any other publication that I could find from the RELAZA2 trial define MRD negativity and they also use the term “advanced MDS” instead of the more precise “high risk MDS” according to either IPSS or IPSS-R. (abstract 1905)

Dr. A.M. Sigmund from Ohio State University presented a retrospective institutional analysis of improvement in survival of AML and MDS patients following allogeneic transplant. The analysis of 900 transplant patients showed improvement in both PFS and OS after 2004. Non relapse mortality (NRM) also improved despite a significant increase in GVHD events. (abstract 2427)

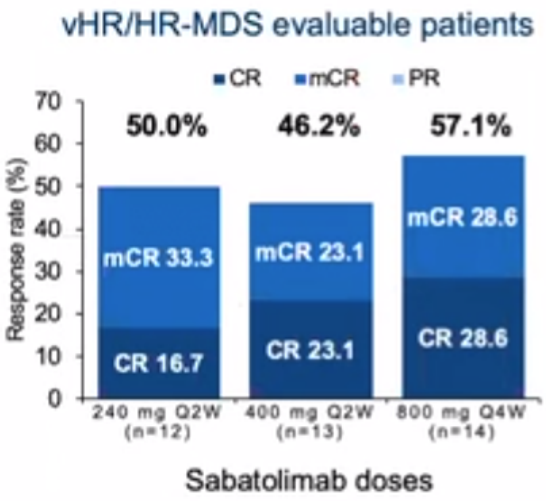


Dr. M. Tobiasson presented a prospective study from the Nordic MDS Group of prediction of relapse after HSCT using so called individualized MRD markers. The aim of the NMDSG14B study is to develop molecular markers for relapse af HSCT to prevent the relapse by emptive intervention. The study plan is shown below. The result of the first part of the study is MRD-assessment, based on patient-specific mutations is feasible with a high sensitivity to predict relapse. However, peripheral blood is less sensitive than bone marrow blood.

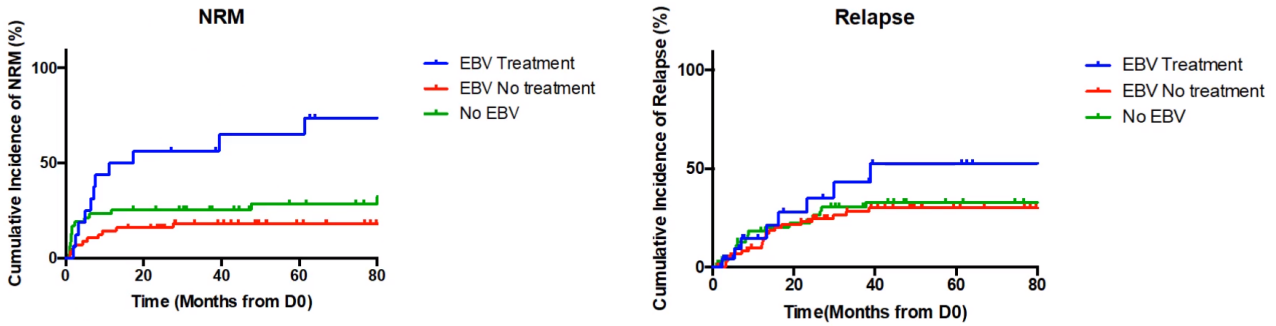


Dr. C. Jouzier reported on a prospective study of transfusion burden in MDS-RS by the Groupe Francophone des Myélodysplasies (GFM). GFM defines low transfusion burden as 2-3 units of Red Blood Cells (RBC) every 8 weeks, and high transfusion burden as more than 4 units of RBC every 8 weeks. Of 100 persons included in the analysis three quarters had iron chelation treatment beside transfusions, which closely corresponded with the number with high transfusion burden. This group also had more hospital admissions, and the largest cause of admission was symptomatic anemia with emergency RBC transfusion. The second largest cause was infections followed by bleeding and cardiac disease. The study also estimated the direct transfusion cost per patient, but unlike a Swedish study did not include indirect cost to society, such as patient transport time and patient lost work time. I think it is very positive that earlier this year both the FDA and EMA approved luspatercept for exactly this group of MDS patients. (abstract 2193)

Dr. A.H. Wei from Monash University in Australia reported on a Novartis sponsored study of dose and safety of sabatolimab (click link to see video of proposed action of this drug) in higher risk MDS and AML. Sabatolimab (MBG453) is a high-affinity, humanized, IgG4 antibody targeting TIM-3, an inhibitory receptor expressed on multiple immune cells and on leukemic stem/progenitor cells and blasts. The figure to the left shows the response of very high risk and high risk MDS patients to this experimental drug, and they are indeed encouraging in this group. The trial established a dose of either 400 mg once every second week or 800 mg once every fourth week in the ongoing phase 2 STIMULUS trials MDS1 (phase 2, under evaluation), MDS2 (phase III, recruiting), and AML1 (recruiting). The abstract for Dr. A.M. Zeidan's presentation of the trials is 1294.

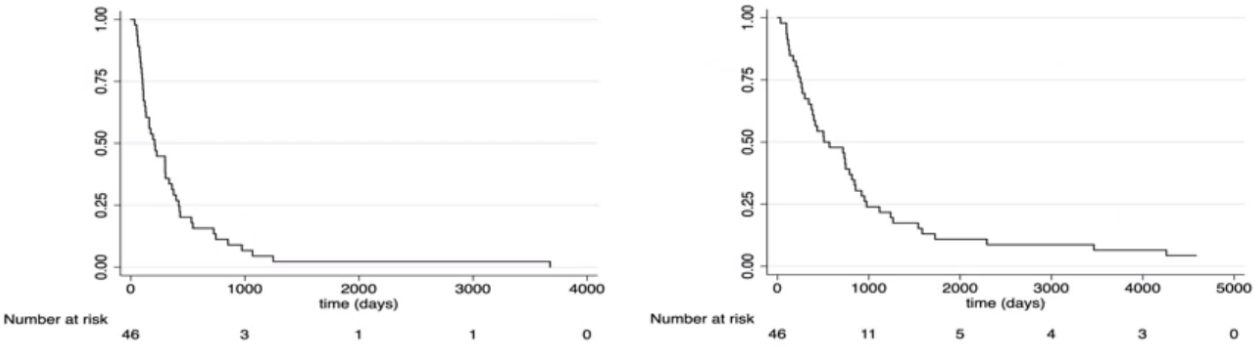


Dr. A.A. Prabahran reported on an Australian study of high risk MDS and AML patients, who had undergone HSCT after T-cell depletion with thymoglobulin, and after transplant developed EBV infection requiring treatment. This group of patient's had significantly higher NRM and relapse compared with persons not requiring treatment for EBV. Unfortunately the study doesn't point to what to do about this problem. (abstract 2374)



Dr. M Della Porta from Humanitas University in Milan, Italy reported on a phase 3 trial on the efficacy and safety of luspatercept versus epoetin alfa (Arenesp) in treatment of anemia in very low, low or intermediate risk MDS according to IPSS-R in patients with or without RS not previously treated with erythropoiesis stimulating agents and who require RBC transfusions. This hence tried to extend the use of Luspatercept to a larger group of MDS patients, than initially approved by the FDA and EMA, and essentially with MDS patients with low risk MDS that have not previously had anything but RBC. The aim of the COMMAND trial is RBC transfusion independence. The trial is currently recruiting, but is not available in Denmark; maybe too few MDS patients on transfusions in Denmark? Last year a pharmaceutical company was attempting to interview MDS patients on transfusions in Denmark, and they only found two people interested in participating in the interviews. (abstract 2198)

Dr. A. Cherait from Hospital St. Louis in Paris, France treated patients with AML after MDS or higher risk MDS with reduced dose AZA (60 mg/m2/day) for 5 days each monthly cycle, and this appears to increase PFS and OS in this group of patients, as seen in the graph below. Note that medium OS from enrollment was almost 17 months - close to 3 years. Unfortunately this was an observational study with no randomization. So what do we learn from it? (abstract 1948)



MDS Education Session at 62nd ASH Annual Meeting

This year's virtual MDS education session was titled “Myelodysplastic Syndromes: What we have and what we want" with the faculty being Dr. Uwe Platzbecker from the MDS Dresden Klinikum, Dr. Hetty Carraway from the Cleveland Clinic, and Dr. Bart L. Scott from Fred Hutchinson Cancer Center. The presentation from the 3 faculty members was available for viewing prior to the Q&A online session on Saturday.

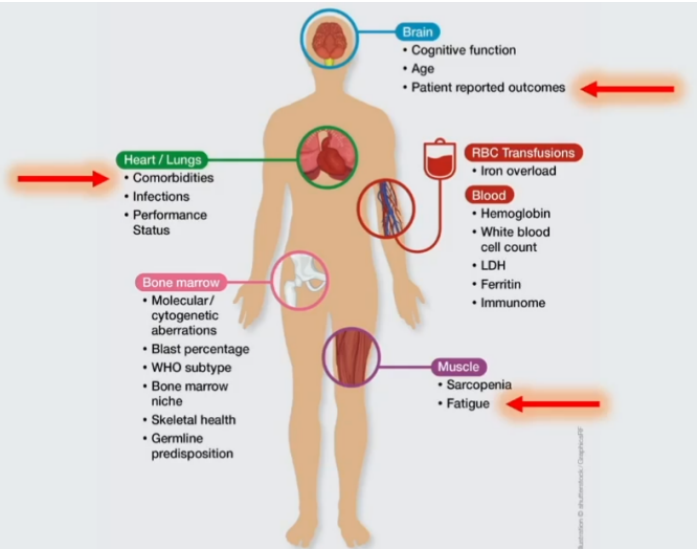
However, the online platform was not perfect and the Q&A session was incorrectly scheduled, so I ended up watching the whole education session on demand. Dr. Uwe Platzbecker talked about risk

stratification in MDS, Dr. Hetty Carraway about therapy for lower risk MDS, and Dr. Bart L. Scott about existing agents, novel agents or transplantation for higher risk MDS.

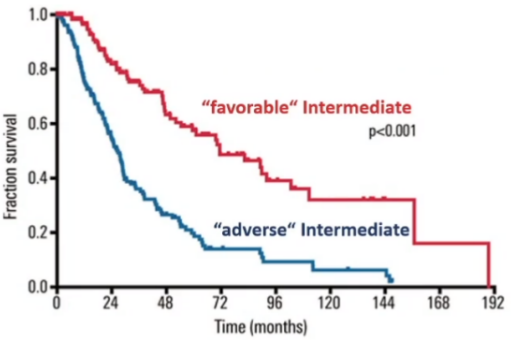
Patient stratification in MDS

Dr. Platzbecker started by showing a map of Germany with 10 MDS centers. Germany has a population of 80 million people, so about 8 million people per MDS center. Denmark has a population of just 5 million. Until November of 2020 we had 7 MDS centers: Copenhagen, Herlev, Roskilde, Odense, Aarhus and Aalborg. If we want to be as attractive for international clinical trials as Germany, then we should have just one center. But even just two would make us a must more attractive for international clinical trials as the current soon to be 6 centers are. Clinical trials are crucial to at least higher risk MDS patients, as we see in Dr. Scott's presentation.

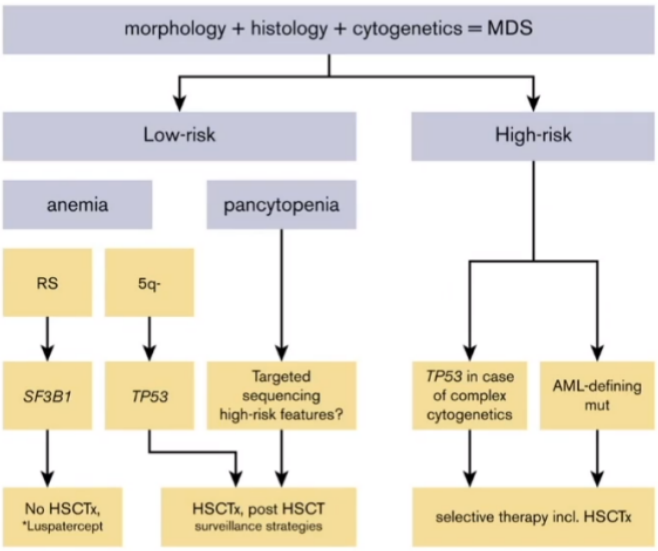
Dr. Platzbecker's presentation was subtitled "How a puzzle may become a map". MDS is challenging because of disease diversity, but the majority of MDS patients are lower risk, and diagnosis is based on morphology and cytogenetics. According to the latest WHO MDS classification include specific subgroups for adults: MDS-SLD, MDS-RS, MDS-MLD, MDS-EB, MDS-U og MDS del(5q), and one group for children: Refractory cytopenia of childhood (RCC). This classification should in my view be used both in clinics and in clinical trials going forward. But unfortunately the worldwide adaptation by doctors, who are not MDS specialists is slow.



However, when deciding on treatment of an MDS patient, after the disease has been classified the MDS specialist has to take into account the whole patient as seen in the illustration here (Source: ASH Educational Book 2020). In this process Della Porta's MDS-specific comorbidity index (MDS-CI) could be used, as it clearly shows increased risk of death - especially with more than one comorbidity. Currently MDS patients are divided into 5 groups using the IPSS-R: Very low, low, intermediate, high and very high. To quantify other information in the figure one could use FACIT-Fatigue scores and the MDS specific frailty scale recently published. However, currently the IPSS-R is the standard for stratification of MDS patients. However, data published two years ago clearly indicates that the intermediate group can be divided into favorable and adverse, as seen in figure below. Other studies by Greenberg et.al show that age at time of diagnosis also matters. So it appears a revision of IPSS-R is warranted. But what about mutations? Do they matter? A study has shown that an SF3B1 mutation is positive news for patients with MDS-RS. And what mutations a patient has matters most for IPSS low and intermediate-1 MDS patients, where the presence of mutations in TP53, EZH2, ETV6, RUNX1 or ASXL1 negatively influence survival, and it also matters how many mutations a patient has. Finally the amount of mutation also matters. More mutations negatively influence survival. So, in my view a revision to the IPSS-R to incorporate information about age

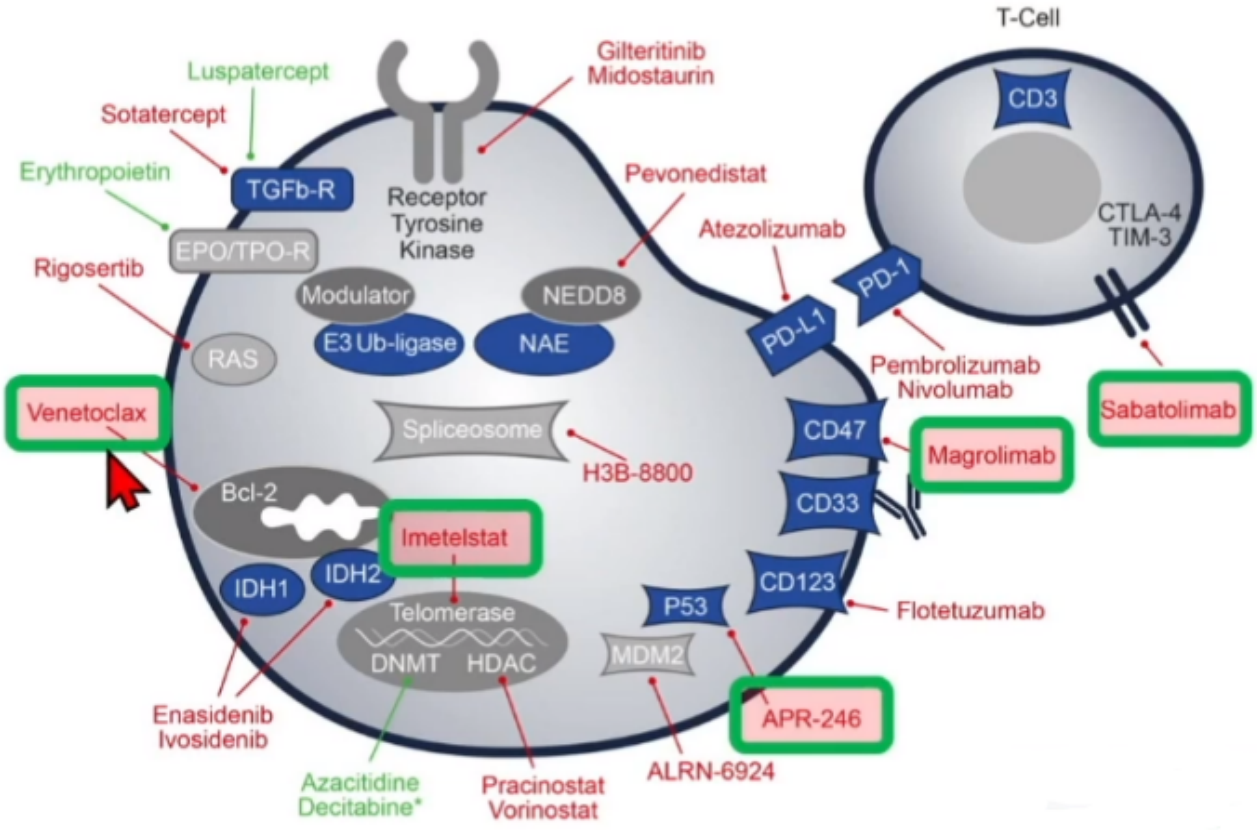


and at least mutations, but properly also comorbidities is urgently called for. We can hope it will be presented at MDS 2021 in Toronto.

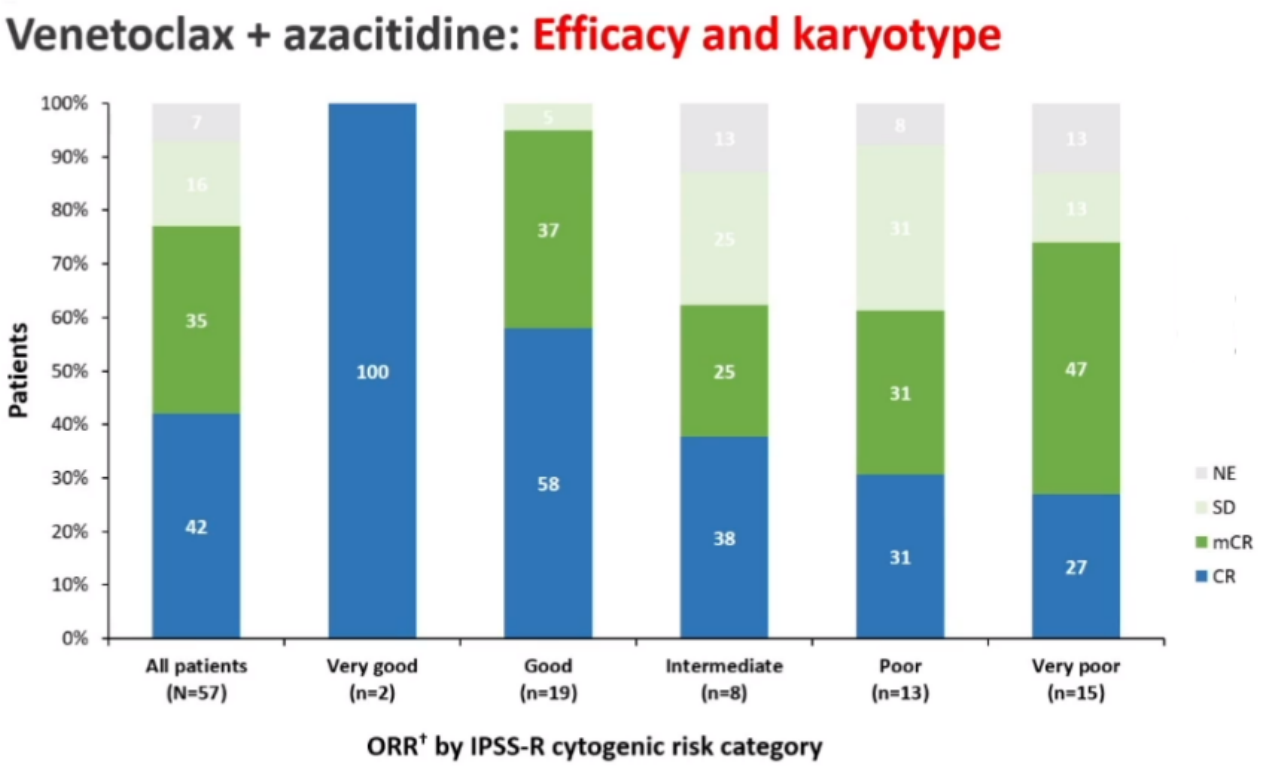


Dr. Platzbecker published the attempt shown here on including mutations in MDS treatment decision making in Blood Advances 2020. The message seems clear, that a patient can have low risk disease according to IPSS or IPSS-R, but the presence or absence of mutations such as SF3B1 with ring sideroblast or TP53 should lead to early consideration of HSCT.

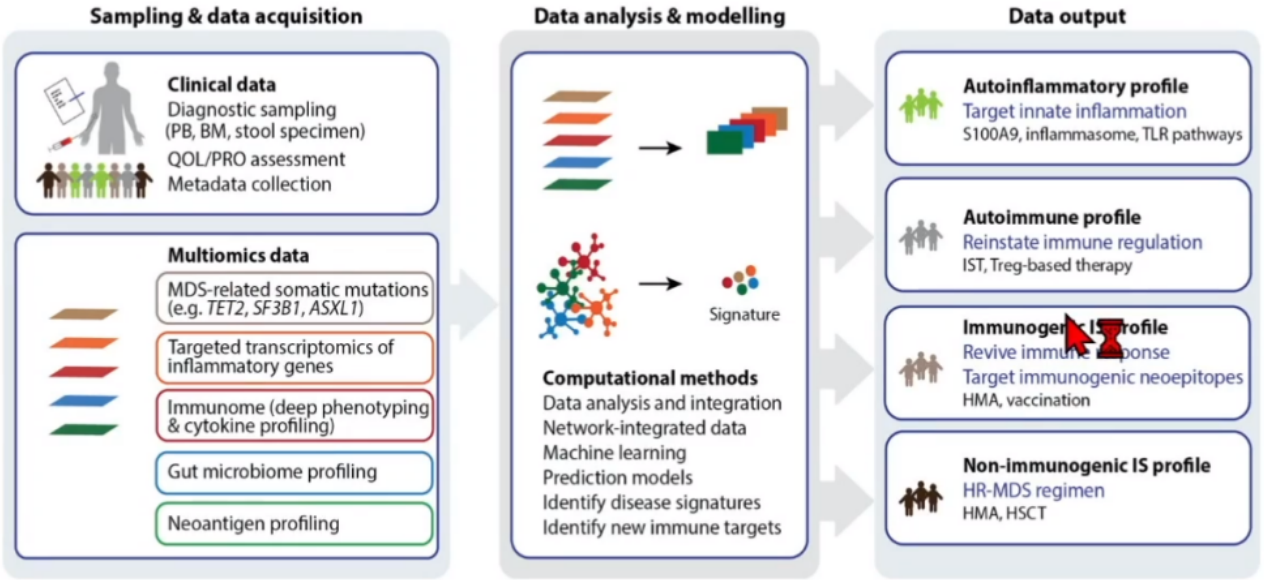
Since the first drug aimed at treating MDS was approved by the FDA in 2007 much has happened, and the treatment landscape is currently changing if not every month, then at least every year with reports on many investigational drugs at both EHA and ASH. The current diagram for approved drugs and drugs under investigation and their attack vectors is shown in the diagram below, where approved drugs are in green and drugs under investigation in clinical trials are shown in red on cell schematic, which also show the genes the different drugs are aiming at:



A very encouraging result of combining venetoclax and azacitidine was presented at EHA earlier this year with a good response shown in all groups of IPSS-R MDS patients, complete response (CR) ranging from 27% in very poor IPSS-R cytogenetic category to 100% in very good, and marrow complete remission (mCR) between 25% (intermediate) and 47% (very poor).



Towards the end of the presentation, Dr. Platzbecker talked about the use of Artificial Intelligence (AI) in connection with stratification of MDS patients and selection of personalized treatment, and presented the following technical overview of the input data (clinical data about the patient and multiomics next generation sequencing data), the computation methods needed for modelling and data analysis, and the output of treatment suggestions.

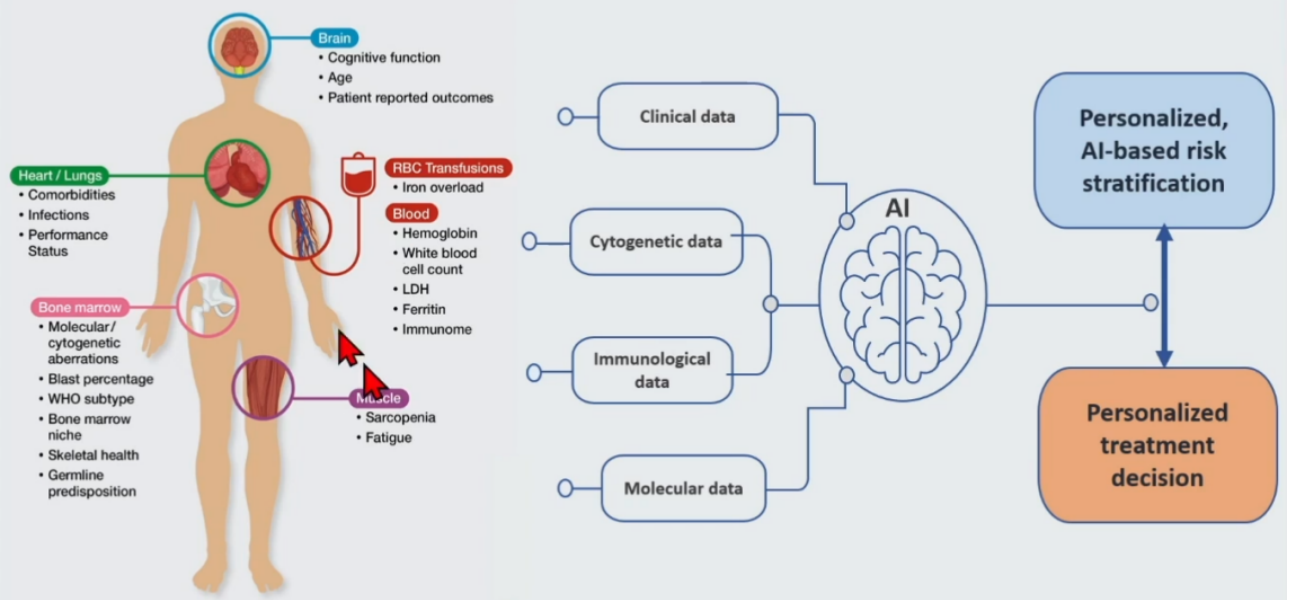


Dr. Platzbecker concluded that,

- current MDS prognostic systems are only an approximation in personal risk stratification.
- putting the information together will require the integration of complex molecular and immunological interactions with clinical variables.

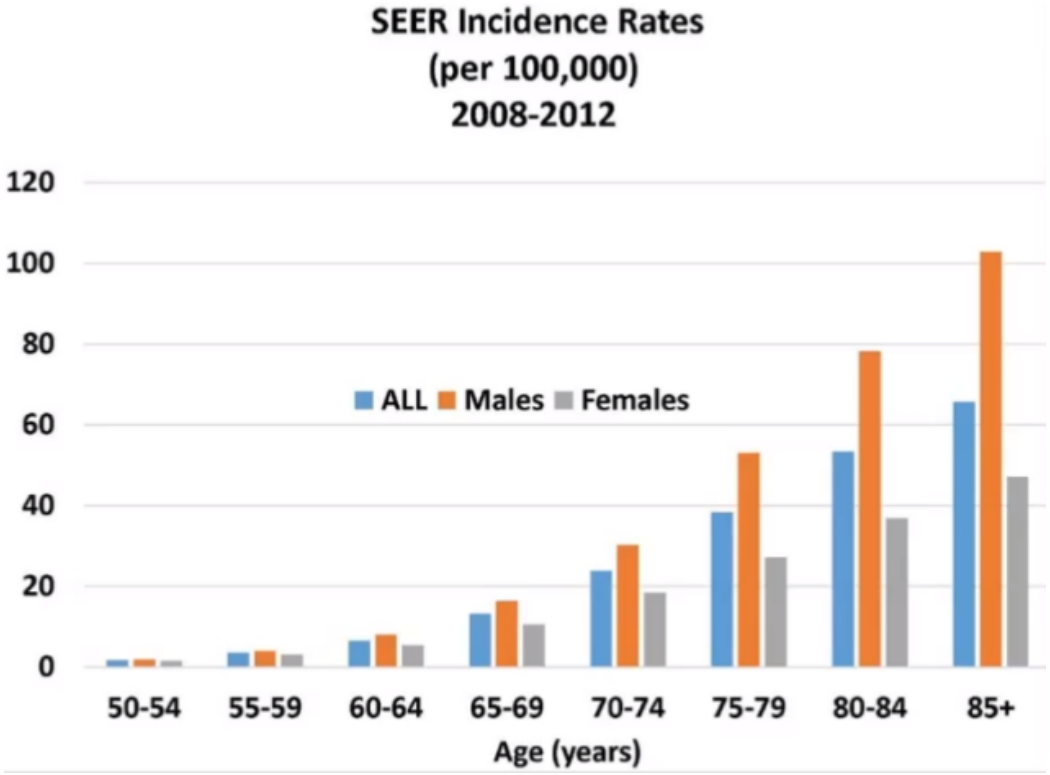
- **An AI-based next generation classification and prognosis system for MDS patients with integration of comprehensive immunological, genomic and clinical information will push patient stratification to the next level**.

and presented the following schematic of such a system:



Therapy for lower risk MDS

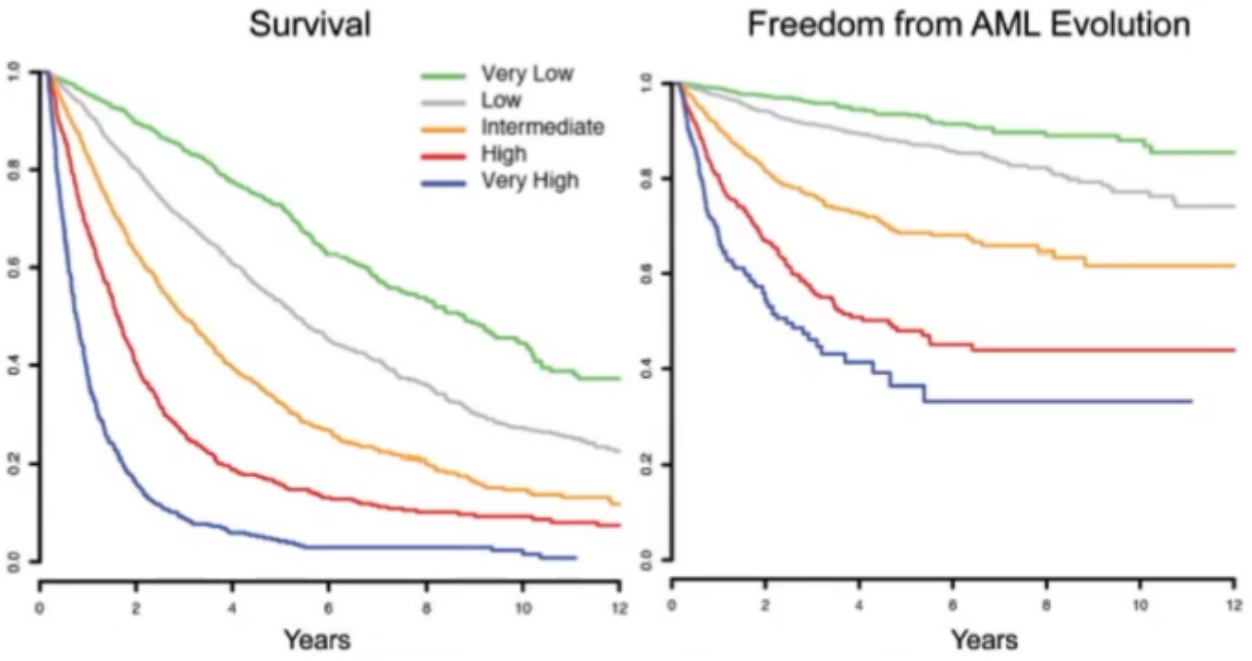
Dr. Hetty Carraway from the Cleveland Clinic talked about treatment of low risk MDS patients.She started by establishing that MDS is a disease of the elderly and as age increases the disease becomes more prevalent in males and in females at least based on the SEER data shown below. In about one out of three MDS patients the disease progresses to AML.



Lower risk MDS is characterised by dysplasia in one or more of the three main cell lines in the blood - red blood cells, white blood cells and platelets, a low marrow blast percentage, moderate

cytopenias in one or more blood cell lines, and - according to Dr. Carraway - relatively good risk karyotype and molecular abnormalities. I tend to disagree with the latter, as several studies - some cited by Dr. Platzbecker - have shown that some molecular abnormalities (mutations) move a low risk patient look more like an intermediate (IPSS-R) or intermediate-2 (IPSS) patient.

Dr. Carraway also classifies lower risk MDS as patients with IPSS score less than or equal to 1 or IPSS-R scores less than or equal to 3.5, and states that the IPSS or IPSS-R score should be supplemented by next-generation sequencing for MDS specific mutations. Dr. Carraway further says that all MDS patients get supportive care, and should be considered for enrollment in clinical trials. Thus in my view we have a huge need for cross-border access to clinical trials, as many MDS patients live in countries or areas of countries with no, very limited or very difficult (travel) access to clinical trials.
For survival and freedom from progression to AML, Dr. Carraway cites Greenberg et.al (2012) and Hellström-Lindbeg et.al (2020) for the data shown in the following figure:



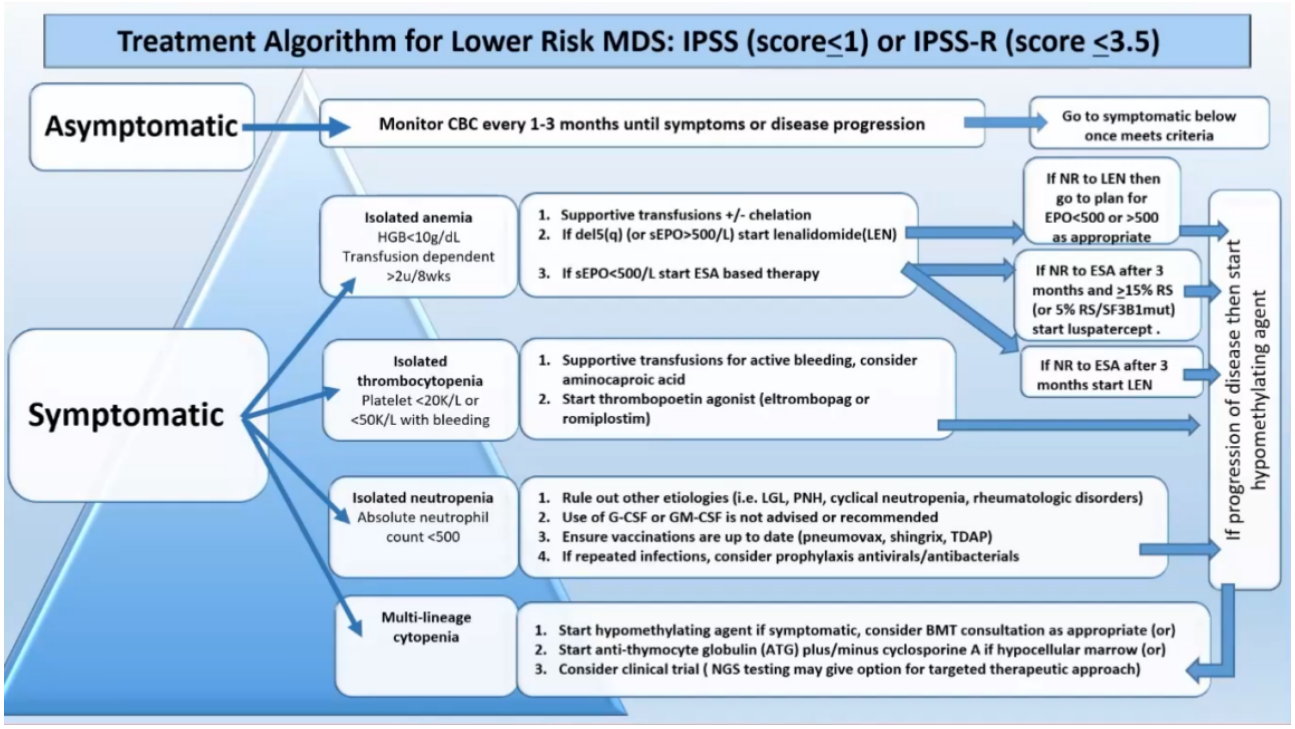
I very much appreciate the positive words in the titles of these two graphs: survival and freedom. Messages such as the ones contained in these graphs, when communicated to patients should emphasize the positive.

Dr. Carraway stated the goal of treatment of lower risk MDS is:

- Supportive care by decreasing symptoms impacting quality of life
    - Transfusion support (RBC, platelet, etc)
    - Chelation treatment to prevent iron overload leading to organ failure
- Improve hematopoiesis and function
    - Growth factor treatment (e.g. Aranesp with/without Neupogen)
    - Decrease infections by treating neutropenia
    - Decrease transfusion burden
- Lower risk of transformation to AML
- Cure

A cure is in my view currently eutopia, since the only potential cure is a HSCT, which has several unpleasant elements, such as death before engraftment, death from GVHD after engraftment, prolonged GVHD, and life long medication post HSCT. The medical literature shows survival historically below 50%, however recent data on HSCT of MDS patients at Rigshospitalet, Denmark suggest survival can be as high as +75% even in a population including men above 70 years and with comorbidities.

Dr. Carraway present the following decision tree for choice of treatment for lower risk MDS patients:



After describing treatment with Erythropoiesis-stimulating agents (ESAs), Dr. Carraway discusses results from a study of Lenalidomide - a product very similar to thalidomide, which caused severe birth defects in the late fifties and early sixties - to MDS patients with del(5q) with or without ring sideroblast. However, the response was clearly better in the group with ring sideroblast. Earlier this year a new drug Luspatercept was approved by both FDA and EMA for MDS patients with ring sideroblasts.

As far as I know a comparison of lenalidomide and luspatercept from a patients perspective, i.e. with focus on patient reported quality of life have not been performed yet, and could help deciding which of these two drugs to give MDS del(5q) patients with ring sideroblasts.

Other treatment options for lower risk MDS patients are Anti-Thymocyte Globulin (ATG). Clinical trials are ongoing in using low dose azacitidine to lower risk MDS patients, who respond to ESAs. Some emerging therapies in lower risk MDS patients are listed in the table on the next page. Both Roxadustat and Imetelstat are in phase 3 trials for lower risk MDS patients without del(5q) and have reported good preliminary results.

Several others are also encouraging, although they are aimed at MDS patients with higher risk disease, such as APR-246 for patients with a TP53 mutation or Ivosidenib for patients with an IDH2 mutation.

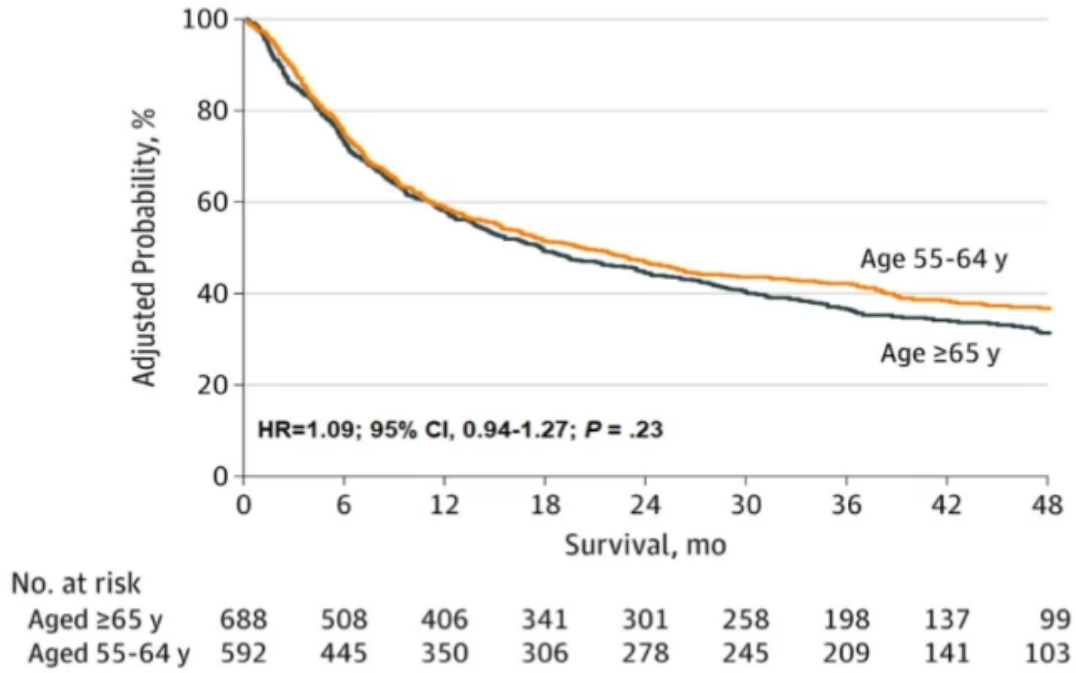
Agent	Mechanism of Action	Route	Patient Population	Single or Combination	Response Rate
Roxadustat	Hypoxia inducible factor (HIF) inhibitor	PO	LR-MDS (non-del 5q) with low Transfusion burden (TB), sEPO <400 U/L	Phase 3 Study ongoing Roxa versus Placebo	Dose finding Cohort results: N=24 HI-E=54%, TI=38% Higher dose TI=78%
Imetelstat	Telomerase inhibitor	IV	LR-MDS (non-del 5q) with high TB and ESA failure	Phase 2/3 IMERGE Study	HI-E=68%, TI for 8wks=42% CR=13%, CRi=10%; myelosupprsn
H3B-8800	Spliceosomal inhibitor	PO	LR-MDS with spliceosomal mutations	Phase 1	HI=14%, no CR/PR PD studies/dose dept splice modltn
APR-246	TP53 modifier	IV	Treatment naïve HR-MDS/AML with TP53m	Phase 2: Combined w/ HMT	RR+75-87%, CR=55% in P2 w/ HMT
Ivosidenib	IDH1 inhibitor	PO	R/R MDS with IDH1m (N=12)	Phase 1 single agent	CR=5/12 and RR=11/12
FT-2102	IDH1 inhibitor	PO	MDS and AML (N=36)	Phase 1 and 2 Single agent +/- HMT	CR/CRi=38% single (N=16) CR=27% combo w/ HMT
Enasidenib	IDH2 inhibitor	PO	R/R MDS with IDH2m (N=17)	Single or combined	1/17=CR and 10/17=RR

I wonder if the low response duration of 18-24 months to ESAs, which Dr. Carraway cites, is due to incorrect classification of some IPSS-R intermediate patients, as referenced by Dr. Platzbecker? I live in Denmark and have met MDS patients with a longer response to ESAs.

Existing Agents, Novel Agents or Transplantation for High Risk MDS

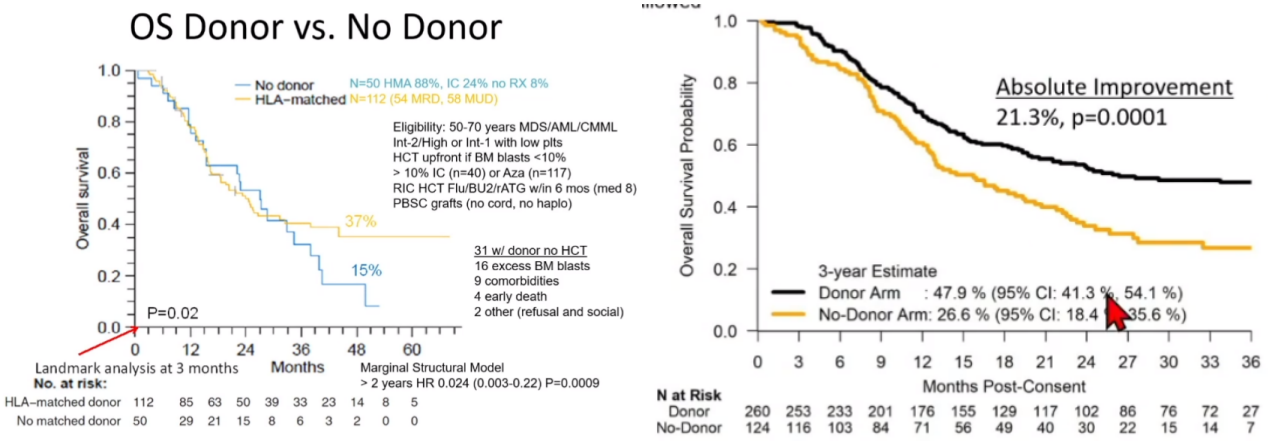
Dr. Bart Scott from Fred Hutchinson Cancer Research Center started the Q&A by stating that first line treatment for higher risk MDS patients should be enrolling them in a clinical trial. Adding that the statement properly meant he would never again be invited to talk at an ASH Education Session, to which Dr. Platzbecker replied that he would still be welcome in Germany.

Dr. Scott started by showing the graph below comparing survival for patients transplanted at age above 65 with patients transplanted with an age between 55 and 64:



Dr. Atallah found that MDS patients undergoing a HSCT at age greater than 65 years had similar survival as patients between 55 years and 64 years. I can add that the experience at

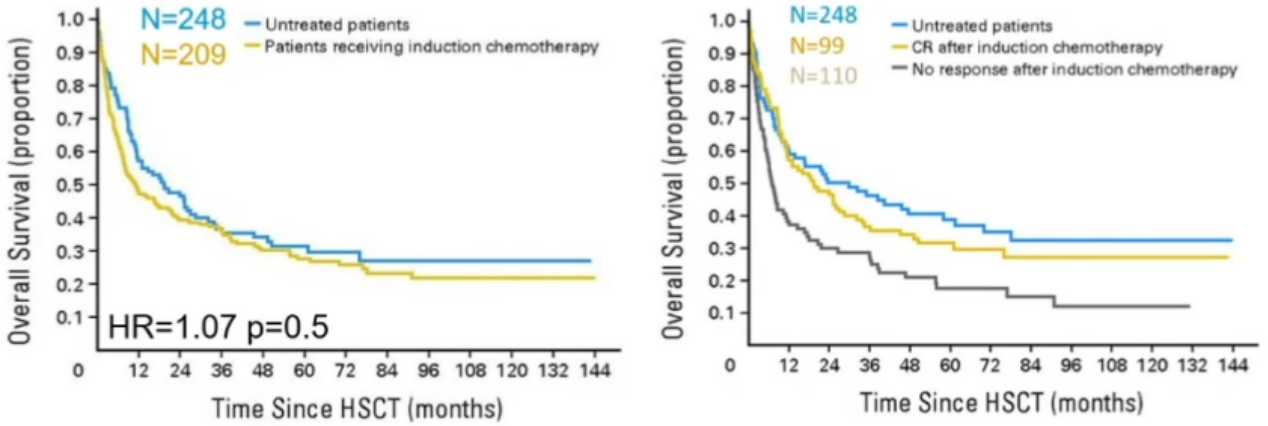
Rigshospitalet in Denmark after introducing a new conditioning regimen for MDS patients in 2017 was that age based on date of birth was not relevant for HSCT outcome and very preliminary data show one year survival above 90% and 3 year survival above 75% (information communicated by leading MDS and transplant doctors at local Danish meetings for MDS patients).
The second question raised by Dr. Scott was if HSCT improved survival, as shown in these graphs



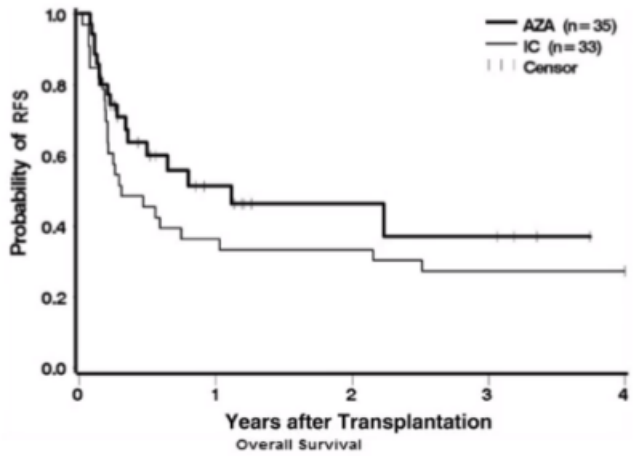
The left graph by Robin et.al (2015) shows that if the patient gets through the first 2-3 years after a HSCT, then there is an advantage. The right graph is a more recent analysis by Nakamura et.al presented at ASH 2020.

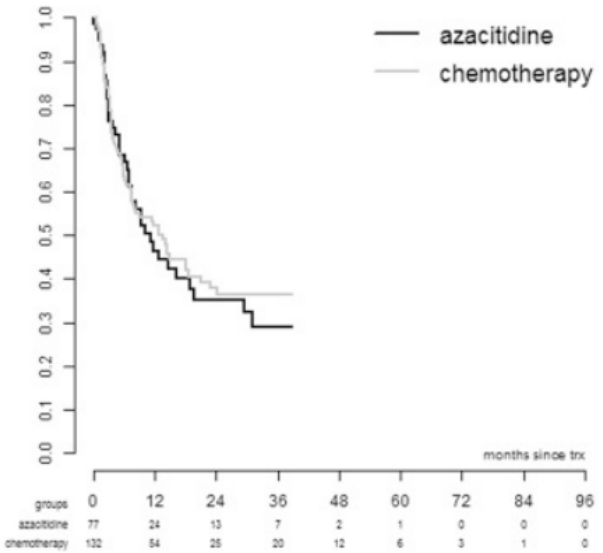
However, I believe the first years after a transplant is a rough ride, and hence more data especially from the Nordic transplant experience for MDS patients in the past 4-5 years is needed in order to properly advise MDS patients about the advantage of HSCT.

Dr. Scott continued by showing data on the influence of pre-treatment before HSCT, e.g. hypomethylating agents (HMA) versus induction chemotherapy (IC).

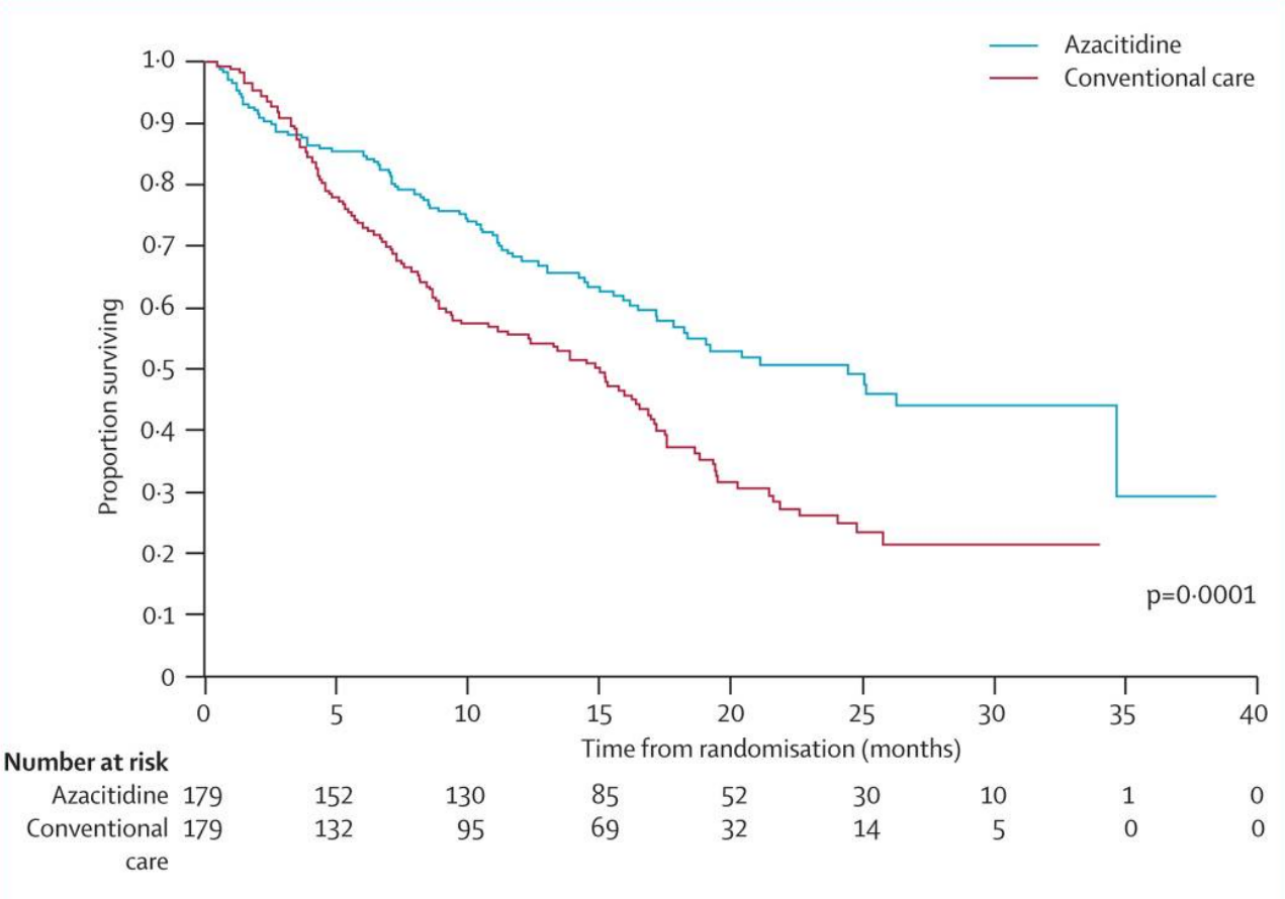


These data from Alessandrino et.al (2013) indicate no survival advantage for patients pre-treated with induction chemotherapy compared to untreated patients. However, a closer look (right graph) indicates that patients with a complete response (CR) to induction chemotherapy performed better after HSCT. As far as HMA versus IC based on Gerds et.al (2012) patients pre-treated with azacytidine have slightly better relapse free survival (RFS) than those pre-treated with induction chemotherapy, but later data by Potter et.al (2016) show no difference.





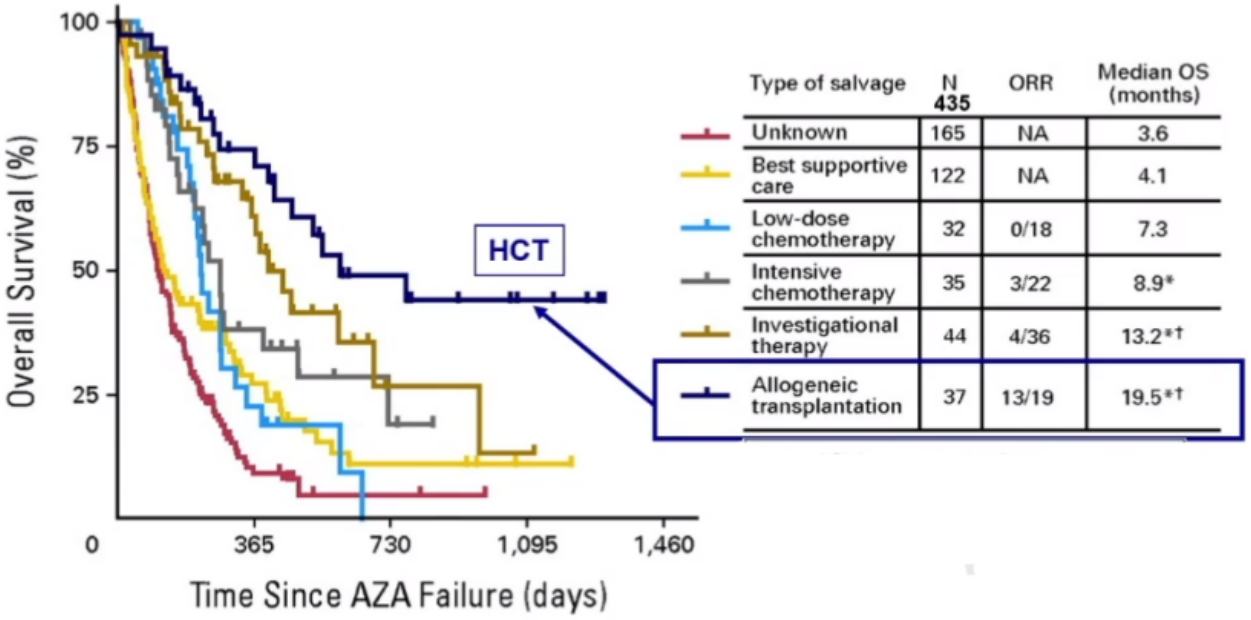
The phase 3 results from the azacytidine trial published by Fenaux et.a (2009) showed the impressive effect of this drug in higher risk MDS patients, as seen in the graph below. However, in the clinic doctors started to see the effect of AZA disappearing after a rather short period compared with results from the trial.



Dr. Scott compared other drugs approved for use in MDS by either FDA and / or EMA. Decitabine has been approved by the FDA for use in MDS, but failed to get approval in MDS by the EMA - see e.g. study by Lübbert et.al (2011), which showed no effect on OS and limited effect on PFS. An oral formulation of decitabine which include some cedazuridine have been shown to be equivalent to IV decitabine in the ASCERTAIN trial - see Garcia-Manero et.al (2020).

Results of a number of combination treatments with preliminary results presented at ASH 2020 were mentioned by Dr. Scott: Venetoclax and Azacytidine, Magrolimab and Azacytidine, APR-246 and Azacytidine, Pevonedistat and Azacytidine. More information about these can be found in the report on oral presentations.

The big question currently with higher risk MDS patients is what to do after HMA failure. There is as seen in the following graph no good options.



Type of salvage	N 435	ORR	Median OS (months)
Unknown	165	NA	3.6
Best supportive care	122	NA	4.1
Low-dose chemotherapy	32	0/18	7.3
Intensive chemotherapy	35	3/22	8.9*
Investigational therapy	44	4/36	13.2*†
Allogeneic transplantation	37	13/19	19.5*†

Essentially, as you can see in the table there are two options for this group of patients: HSCT or clinical trial. Even with these the median OS is between 1 and 2 years.
Dr. Scott finished by talking about the STOP MDS Trial, which have these simple inclusion and exclusion criteria:

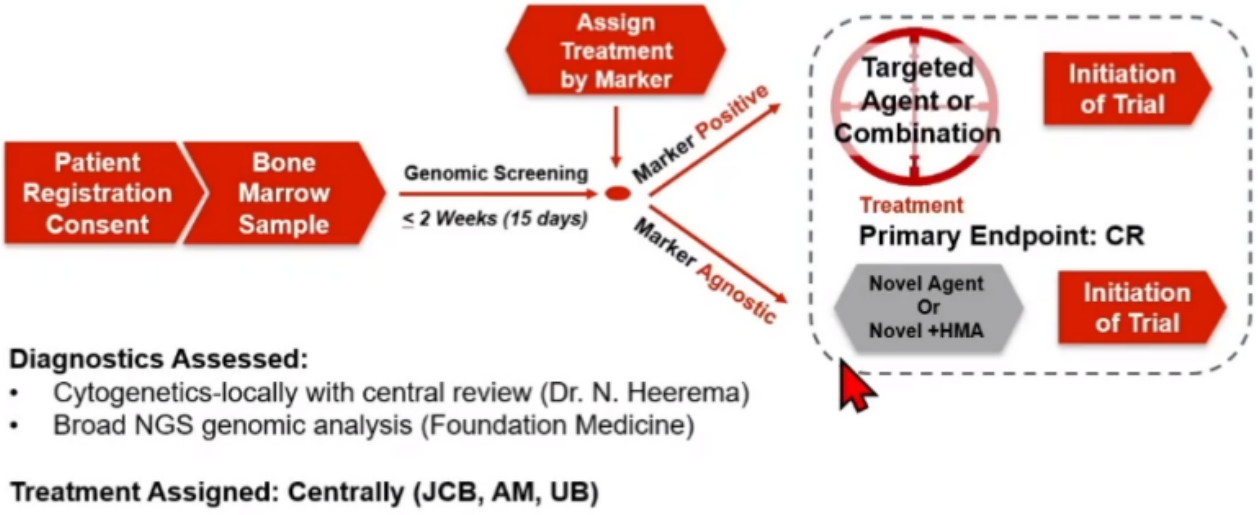
Inclusion Criteria

- Age 18 years or > at time of diagnosis
- Ability to give informed consent
- Group A: untreated intermediate, high, or very high risk MDS (IPSS-R)
- Group B: R/R MDS of any IPSS-R classification after a HMA treatment

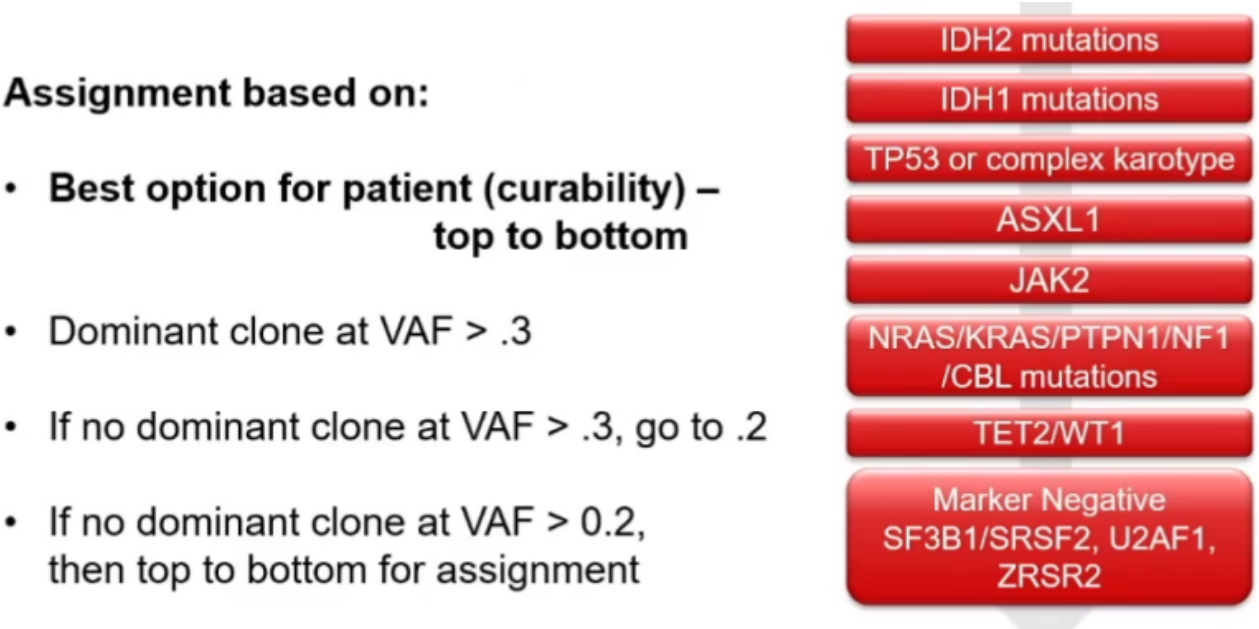
Exclusion Criteria

- Group A: very low or low-risk MDS (IPSS-R)
- Group A: known sole 5q deletion karyotypes
- DIC with active bleeding or signs of thrombosis
- Features that impair compliance with study treatment and follow-up
- Pregnant or lactating females

and the following study design with cytogenetics evaluated locally at participating institutions, but reviewed centrally by Dr. N. Heerema, Department of Pathology, Ohio State University and with a broad Next Generation Sequencing (NGS) genomic analysis by Foundation Medicine:



and with treatment assigned using this schema;



An investigational new drug application (IND) was submitted on November 6th, 2020 with one master protocol and 3 sub-protocols, and the first patient in (FPI) is estimated at February, 2021. Additional studies to be added during 2nd quarter 2021. VAF is an abbreviation for Variant Allele Frequency – a measure of how often a mutation appears in a gene.

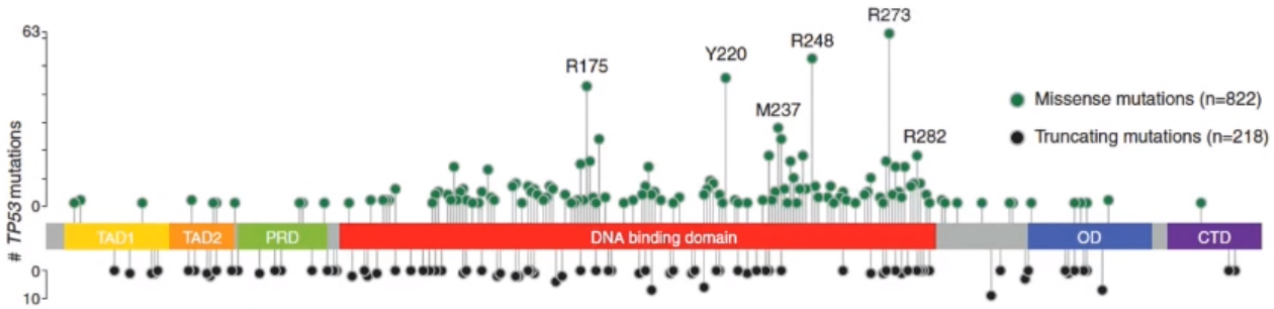
Myelodysplastic Syndromes - Basic and Translational Studies

This session featured 6 presentations. Two of them focused on disease progression from Aplastic Anemia / Paroxysmal nocturnal hemoglobinuria AA / PNH to MDS / AML and from MDS to sAML (secondary AML).

Missense mutations dominating in mice studies

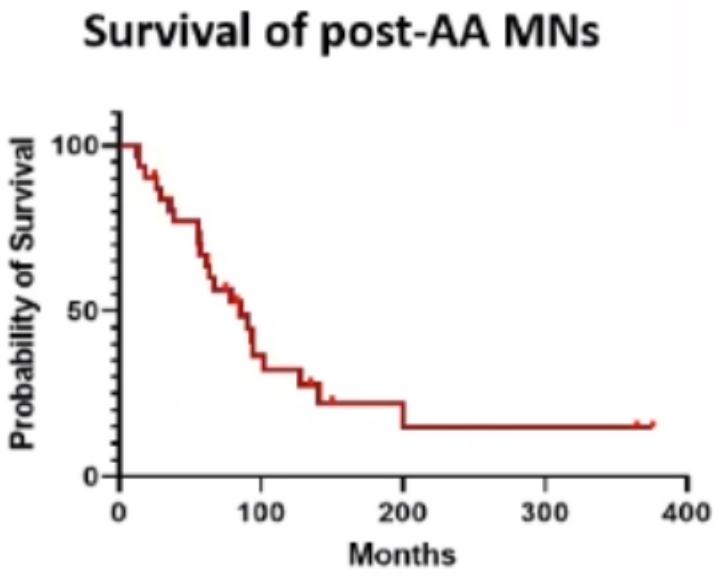
The second presentation in this oral session was titled "Mutant TRP53-R172H Has Gain-of-Function or Dominant-Negative Effects in Response to Different Hematopoietic Stressors in Mice" by Dr. Tanzir Admed, who had performed a study in mice of the mutation TRP53-R172H, which corresponds to the human TP53-R175H mutation. TP53 mutations are common in MDS and

AML, and are mostly missense mutations, as seen on the following graph below. A missense mutation is a mistake in the DNA which results in the wrong amino acid being incorporated into a protein because of change. In this study it is a G to A amino acid substitution. Unfortunately, I don't have the knowledge of animal studies to further comment on abstract 407.

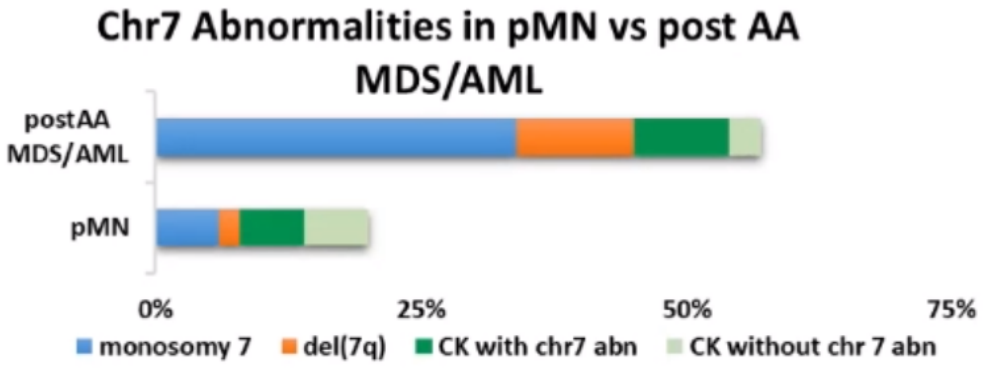


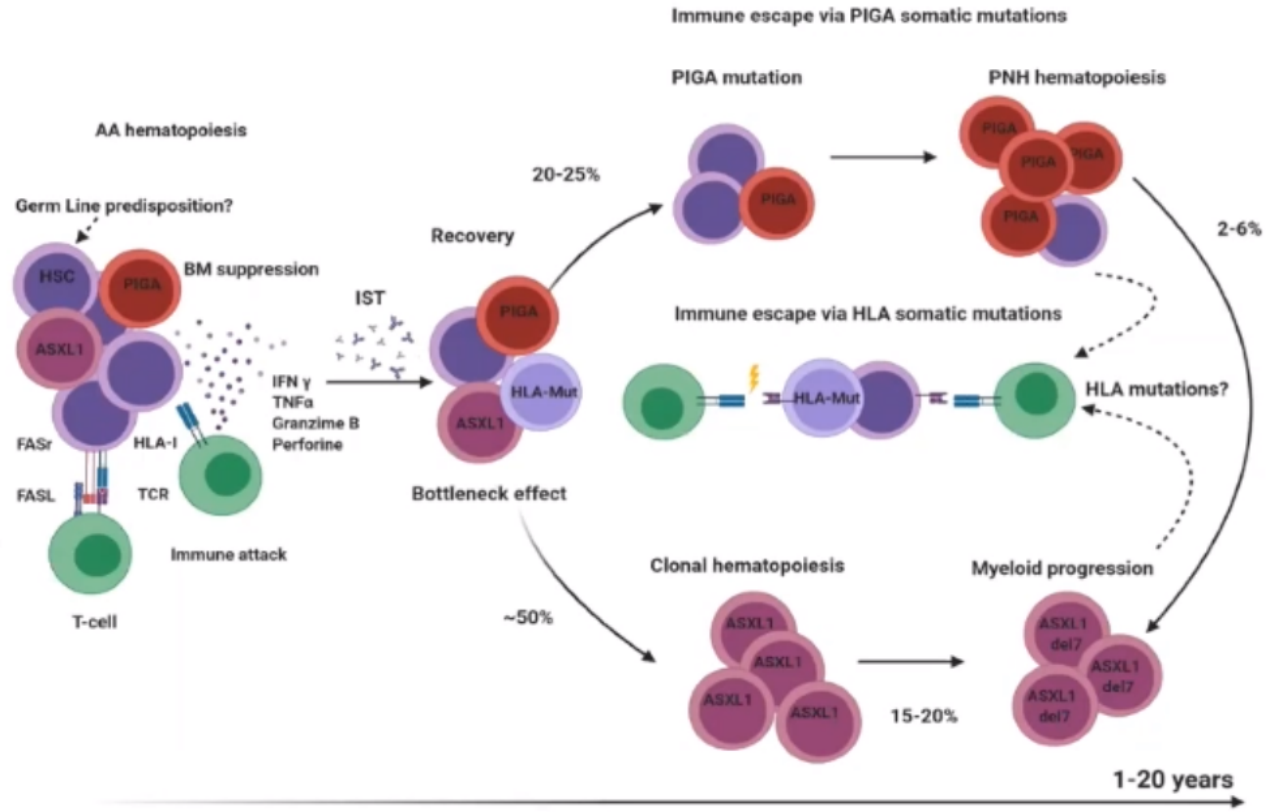
Genomic landscape in AA/PNH progression to MDS/AML

The third presentation was titled "The Genomic Landscape of Myeloid Neoplasms Evolved from AA / PNH" and given by Dr. Carmelo Gurnari from Italy. He told us that 15-20% of aplastic anemia patients over 10 years develop secondary MDS or AML with poor survival, as seen here. MNs is shorthand for myeloid neoplasms, e.g. MDS or AML. However, even after 25 years the probability of progression is only 25%.



Dr, Gurnari described a study comparing the genomic landscape of a large group (654) of patients with primary MDS or AML with a small group (22) of patients who had progressed to MDS or AML from AA, and found large differences in chromosome 7 mutations between primary MDS or AML and secondary MDS or AML after progression from AA. And significant molecular differences: TET2 and SF3B1 mutations were less common in secondary MDS or AML, and mutations in SETBP1, ASXL1 and BCOR were more common in secondary MDS or AML.

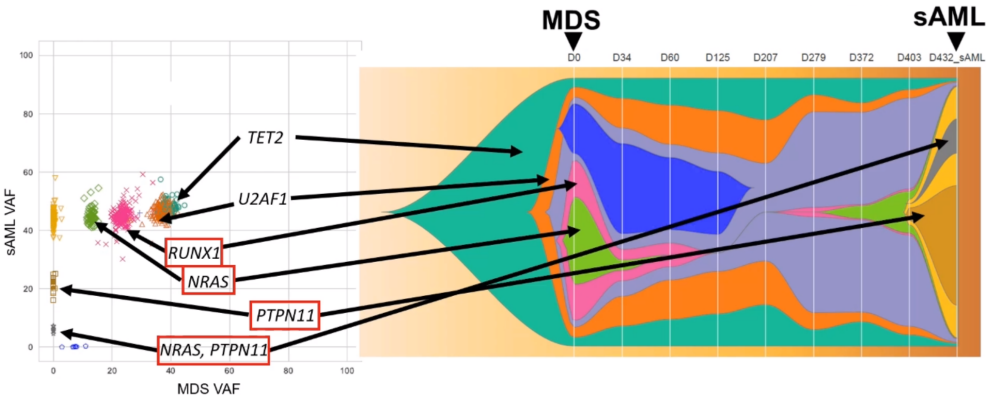




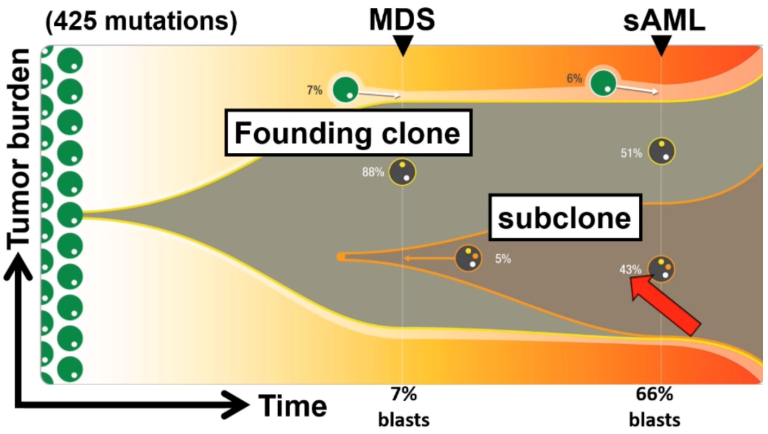
Dr. Gurnari also analyzed the evolution of the mutations, which showed a significant increase in Clonal Hematopoiesis of Indeterminate Potential (CHIP)-related mutations. However, more long term follow data are needed to identify risk factors.The above picture shows evolution from AA to PNH and myeloid malignancies. Notice that this is a slow process. The time scale at the bottom covers 20 years.

Genomic mutations in MDS progressing to sAML

The fourth presentation titled "Signaling Gene Mutations Are Characterized By Diverse Patterns of Expansion and Contraction during Progression from MDS to Secondary AML" was given by Dr. Andrew Menssen from Washington University School of Medicine in St. Louis. Dr. Menssen started with picturing the clonal evolution of MDS and its progression to AML. Today the difference between MDS and AML has been arbitrarily set at 20% blasts in the bone marrow. In older days, i.e. in the 1900's it was equally set at 30% blast. I sincerely hope that the MDS community soon will use a better tool to distinguish between MDS and AML than blast percentage. A decade ago I knew an American MDS patient, whose blast percentage occasionally shot up in the thirties, and with sometimes more than 5% difference between two types of measurements. Read more about this issue in Dr. Michael Heuser's guest editorial "High risk MDS and AML: One or two diseases?" in MDS News volume 25 Issue 1.

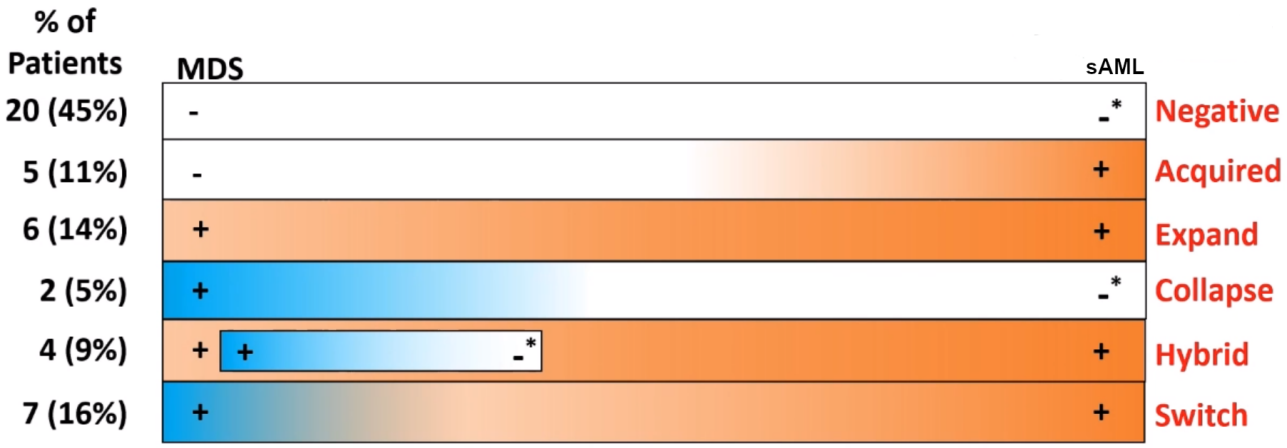


Dr, Menssen explained that mutations in signaling - e.g. receptor tyrosine kinases - and transcription factor genes are more common in secondary AML than MDS, which could indicate a role in disease progression. In order to better understand a possible role, paired samples from the same patient from the MDS phase of the disease and from the sAML phase of the disease are needed to possibly define markers for MDS progression. He therefore analyzed paired samples from 44 MDS patients who had progressed to sAML. The above graph of sAML VAF versus MDS VAF indicates that some mutations don't appear until disease progression, such as PTPN11 or NRAS, PTPN11 that both have 0 MDS VAF. VAF is an abbreviation for Variant Allele Frequency - read more about this in "Current practices and guidelines for clinical next-generation sequencing oncology testing". Others such as RUNX1 and NRAS appear to present in MDS and then disappear only to come back as the disease progresses.



Further analysis showed that transcription factor gene mutations occur before signaling gene mutations, and multiple signaling gene mutations rarely occurred in the same cell. This according to Dr. Menssen indicates that signaling gene mutations are redundant or detrimental to leukemia cells. Unfortunately the clonal evolution is not the same across the patient cohort analyzed in this study as illustrated in the above graph. Signaling gene mutations are mutations that lead to unrestricted cell growth, and transcription factor gene mutations work to turn certain genes on and off.

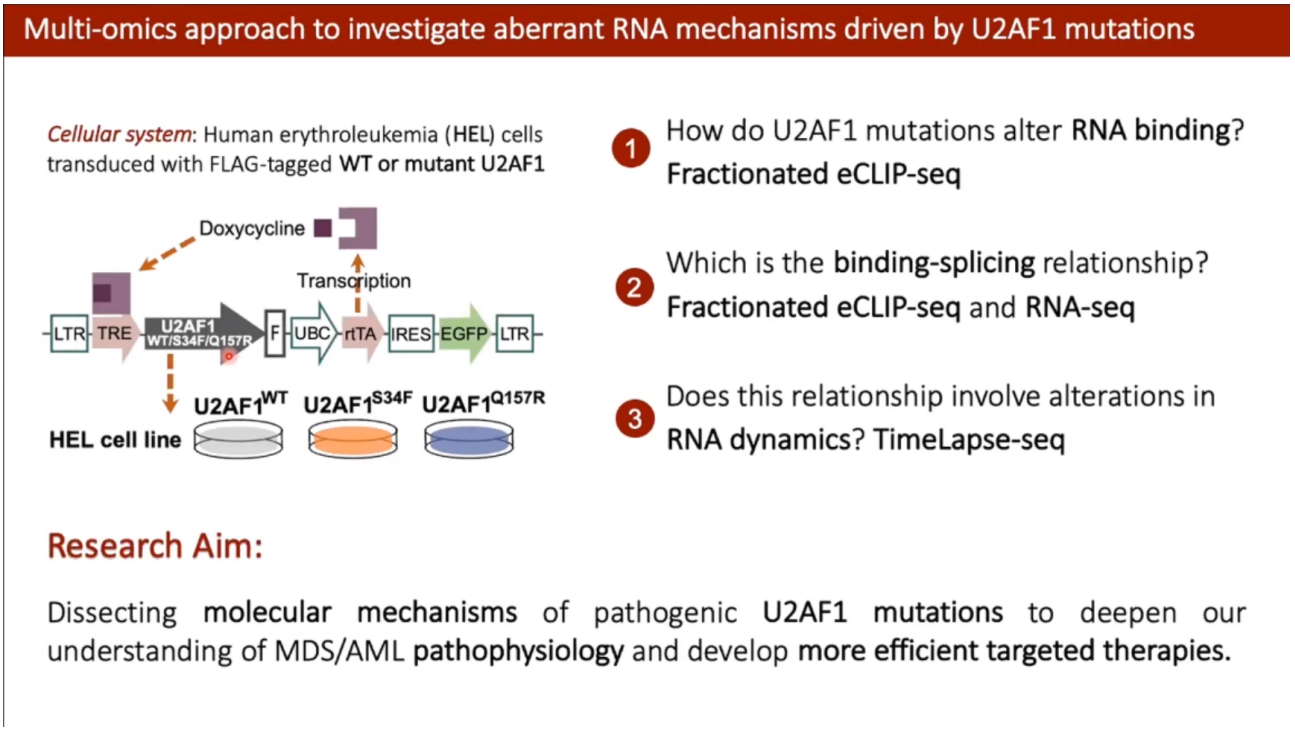
Dr. Menssen concluded that signaling gene mutations are present in nearly half of the MDS patients who progress to sAML, and suggest that signaling genes are a driver of MDS progression to sAML. These signaling gene mutations could be a possible biomarker for which MDS patients will progress to sAML.



Complex Splicing Alterations and Kinetics in Myeloid Malignancies

The fifth presentation in the session on basic and translational studies was "High-Resolution Binding Atlas of U2AF1 Mutants Uncovers New Complexity in Splicing Alterations and Kinetics in Myeloid Malignancies" given by Dr. Giulia Biancon from Yale University School of Medicine. She investigated at the molecular level how U2AF1 mutations disrupt ordered splicing from binding to

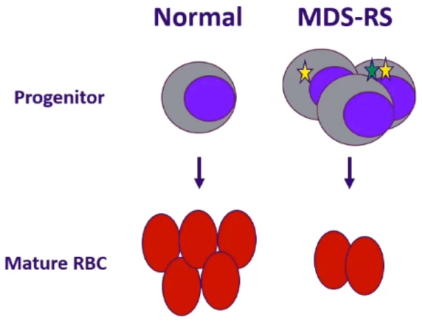
recruitment of cooperating messenger Ribonucleic Acid (RNA) binding proteins and ultimately splicing kinetics using fractionated enhanced crosslinking immunoprecipitation with deep RNA sequencing.
However, basically this is beyond my level of understanding of next generation sequencing and molecular biology, so I just enjoyed Dr. Biancon's impressive slides. This one explains which system is studied, which forms of sophisticated analysis tools are employed and why as well as the

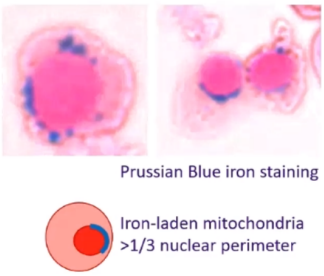


aim of the work: To develop more efficient targeted therapies based on a deeper understanding of MDS / AML pathophysiology by dissecting molecular mechanisms of pathogenic U2AF1 mutations. But I have no idea what fractionated eCLIP-set, fractionated eCLIP-seq and RNA-seq or TimeLapse-seq are except that they are methods at the very frontline of molecular medicine research.

Cause of Ring Sideroblast Formation in SF3B1-mutant MDS

The sixth presentation was titled "Coordinated Mis-Splicing of Multiple Mitochondrial Iron Metabolism Genes Causes Ring Sideroblast Formation in SF3B1-Mutant MDS" by Dr. Courtnee Clough from University of Washington. The basic idea is that in MDS-RS the SF3B1 mutation has a high mutation frequency compared to other hematologic cancers such as Chronic Myelomonocytic Leukemia (CMML), MDS (non-RS), sAML, AML and Chronic Myelogenous Leukemia (CML). This high presence of SF3B1 mutations in MDS-RS suggests a causal connection between SF3B1 mutation and ring sideroblast (RS).

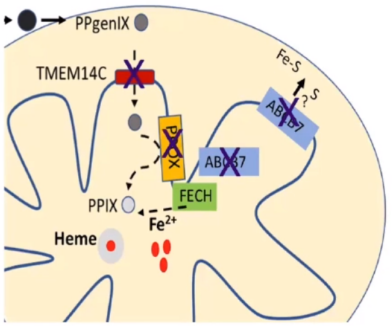


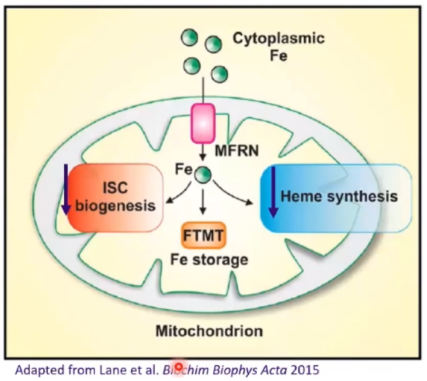


To investigate the mechanism by which SF3B1 mutations cause RS formation the group established an iPSC model of MDS-RS. iPSC is an abbreviation for induced Pluripotent Stem Cell; read more about iPSC on UCLA. The iPSC model of MDS-RS

recapitulates mutant SF3B1 mediated mis-splicing and in vitro ring sideroblast formation. MDS-RS is defined by ineffective erythropoiesis and ring sideroblast formation. The following graph illustrates this.

On the right side is a microscopic picture of blood smear with Prussian Blue iron staining. A schematic of the generation of the MDS-RS model is seen below. It starts with the actual MDS-RS patient cell, that is manipulated in the lab to create the cell used in vitro.

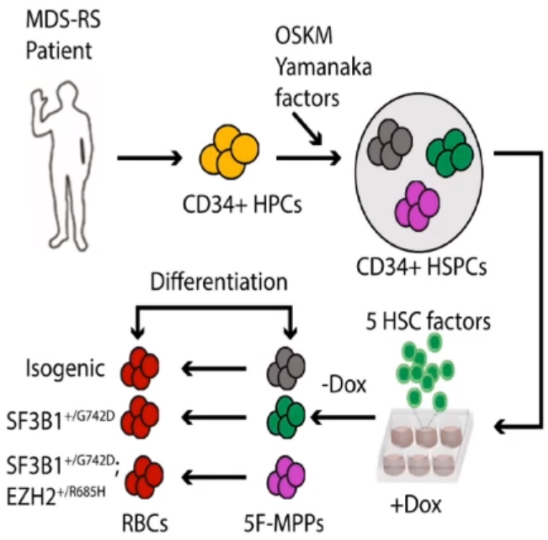




Adapted from Lane et al. *Biochim Biophys Acta* 2015

Using the model the authors found that RS formation increased during terminal erythropoiesis in SB3F1 mutant cells, but not in wild type cells.

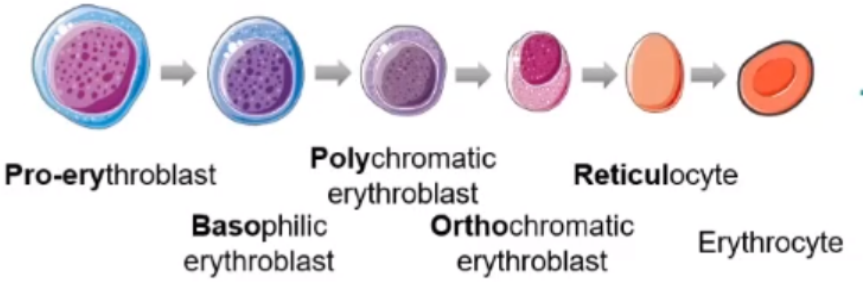
Further experiments were performed to investigate the role of mis-splicing events in ring sideroblast formation. These showed that overexpression of the THEM14C, PPOX and ABCB7 involved in hemoglobin synthesis partially restored RS formation.



Overall the finding confirms the hypothesis that mis-splicing of mitochondrial iron metabolism genes causes RS formation, suggesting that RS formation in MDS is a multigenic event caused by coordinated but incomplete mis-splicing of several critical iron metabolism genes. The following graph is a schematic representation of the findings.

I wonder if the approach described here could be used to create cell models of other MDS disease subtypes. I believe that is the part of the approach of the Program in Translational Hematology established as a cooperation between University of Copenhagen and Rigshospitalet.

Unfortunately I was late for the first presentation in this session given by Dr. Rashmi Kanagal-Shamanna from the MD Anderson Cancer Center in Houston, which was also about SF3B1 mutations in MDS with the title "*SF3B1*-Mutant Myelodysplastic Syndrome with Ringed Sideroblasts (MDS-RS) at the Single-Cell Level". She performed single-cell RNA sequencing of HSPC - hematopoietic stem and progenitor cells - from 2 healthy adults and 5 MDS-RS untreated patients with SF3B1 mutation, and found the mutation created numerical change in the HSPCs. Similar analysis of bone marrow mononuclear cells showed increased erythroblasts and decreased B lymphocytes in the MDS-RS patients. The SF3B1 mutations affect terminal erythroid differentiation in all the steps from pro-erythroblasts to reticulocytes, and HMA treatment promotes differentiation.

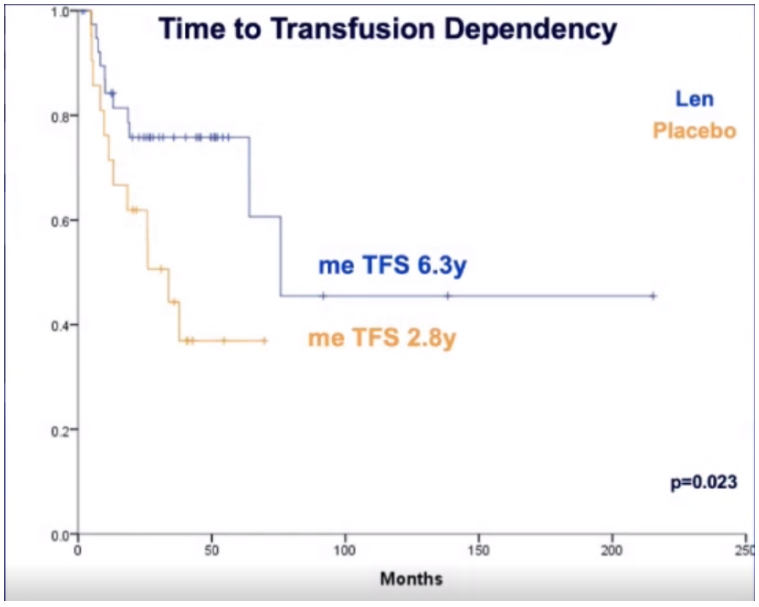


MDS Clinical Session

The MDS clinical session on Monday featured six presentations and was subtitled “Personalized clinical-decision tools and treatment of lower risk myelodysplastic syndromes”. For me the highlight of this session was the two presentations aiming at improving diagnosis using machine learning. I think this is an area with a high potential, so hematologists can focus more on treatment selection.

Lenalidomide prolong time with Transfusion Independence (TI) for MDS Del(5q) patients

Dr. Félix López Cadenas presented an interim analysis of effect and safety of lenalidomide from the European Sintra-REV trial titled “Phase 3 Study of Lenalidomide (LEN) Vs Placebo in Non-Transfusion Dependent (TD) Low Risk Del(5q) MDS Patients - Interim Analysis of the European Sintra-REV Trial”. The analysis showed that lenalidomide - a thalidomide related drug - delayed and reduced transfusion dependency in this group of patients based on analysis of 61 participants as shown in graph below. However, it should be noted that at time analysis 35 patients had left the study for various reasons.

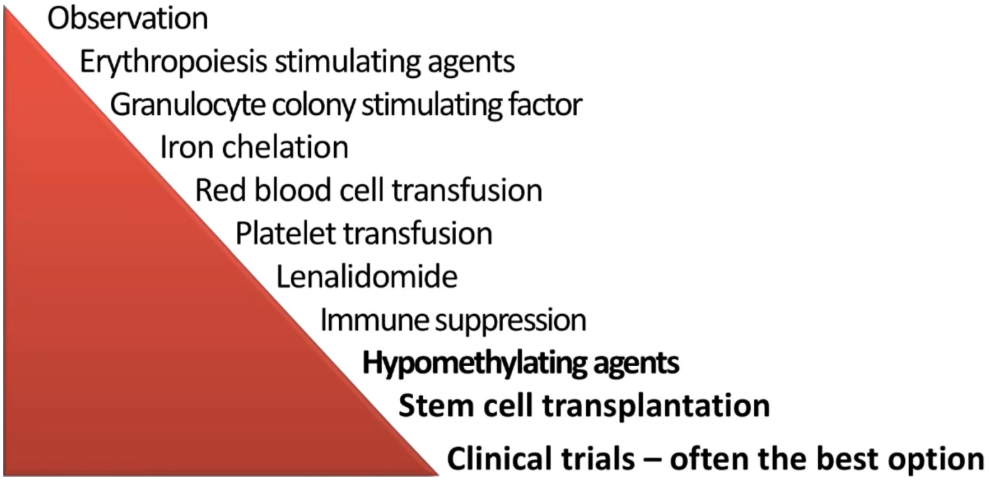


The doctors judged lenalidomide to be well tolerated, as there were few grade 3 or 4 adverse events. Also lenalidomide did not appear to increase risk of progression to AML.

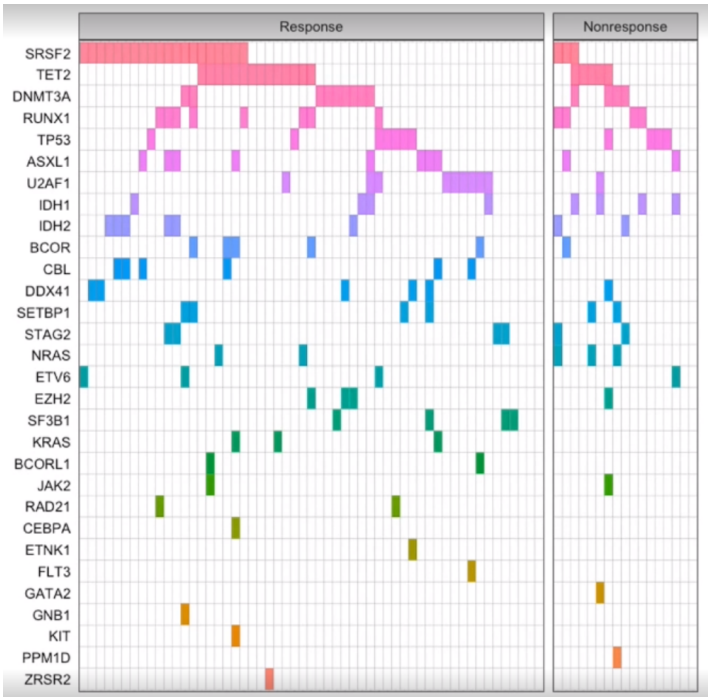
The interim analysis conclude, that early treatment of MDS Del(5q) patients with low doses (5 mg) lenalidomide prolong the time to and decreased the risk of transfusion dependency, that almost ¾ of the patients reached responses, and that more than ⅘ reached cytogenetic response, and the drug had an acceptable safety profile. However, the impact of lenalidomide on long term outcome is not yet known.

Adjunctive agents could improve AZA

Dr. Soo Park from Moores Cancer Center in San Diego talked about “DNA Methylation Analysis before and during Treatment with Azacitidine Plus Pevonedistat or Azacitidine Alone in Patients with MDS, CMML, and AML Previously Untreated with Hypomethylating Agents”. He started by showing the following diagram of treatment options:



Dr. Park's focus is clearly High Risk MDS, as evident by the statement at the bottom of this heraci of treatments: "Clinical trials - often the best option". He continued with data from the AZA-001 trial indicating that time to first response with azacitidine could be more than a year before going on to his focus: Predicting DNA methylation response and combining azacitidine with pevonedistat in a phase 2 trial - a precursor to Takeda's PANTHER phase 3 trial, and first showing the mutation profile of responders versus non-responders in a trial with both MDS, CMML and AML patients. As you can see there is not a single mutation that distinguishes responders and non-responders in this group of patients. Each column represents a patient. So the first responding patient has two mutations: SRSF2 and ETV6. There are also patients without mutations in the panel used in the analysis.



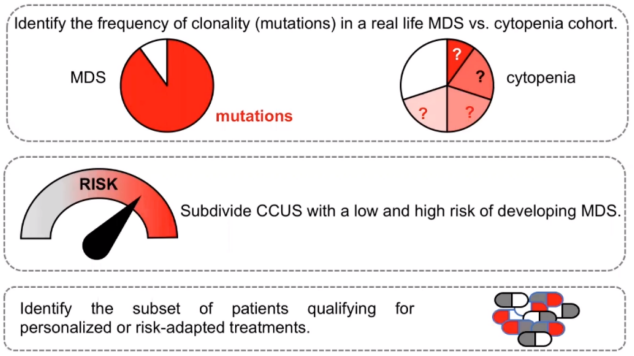
Dr. Park concluded that demethylation patterns early during HMA treatment correlate with eventual response, and complete response is associated with the greatest degree of methylation. Furthermore responders are more likely to have greater demethylation reflecting greater effective AZA exposure, and pevonedistat intensifies demethylation in responders. He calls for investigation of HMA dose escalation in patients with early inadequate demethylation as well as using adjunctive agents.

Molecular Genetics in Diagnosis and Prognosis of Clonal Cytopenia of Undetermined Significance (CCUS) and MDS

Dr. Constance Baer from the Munich Leukemia Lab (MLL) in Germany gave the third presentation in the clinical session on an analysis of molecular genetics in 576 CCUS and MDS patient's titled "The Potential of Molecular Genetic Analysis for Diagnostic and Prognostic Decision Making in Clonal Cytopenia of Undetermined Significance (CCUS) and MDS – a Study on 576 Patients". The

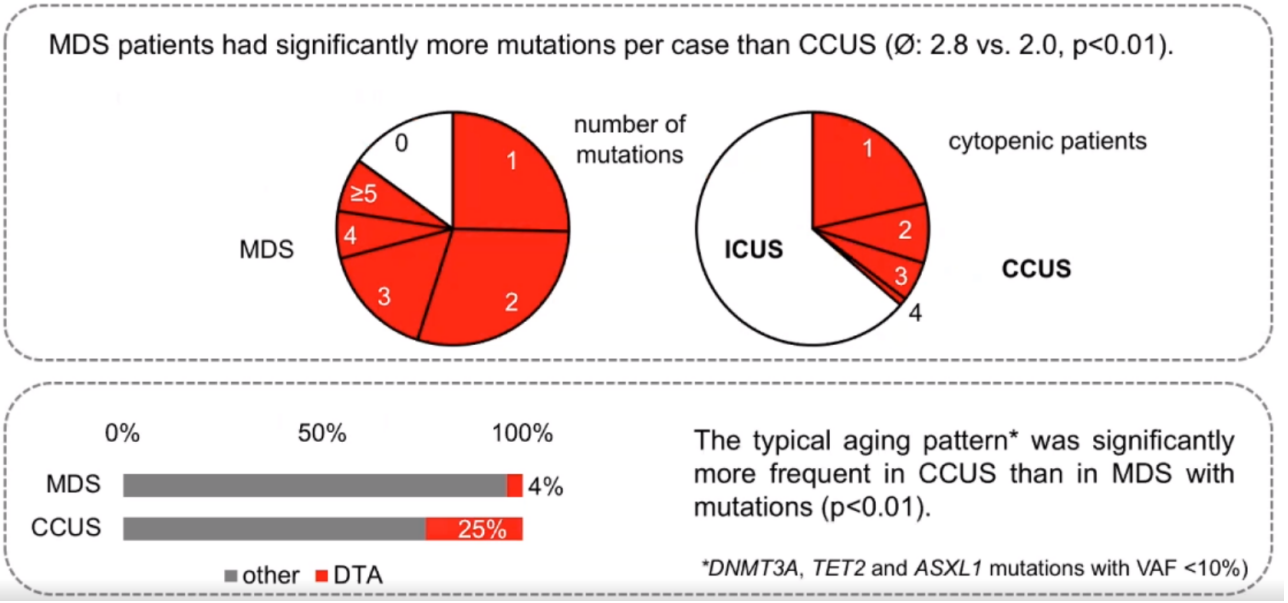
current challenges in CCUS, MDS and AML are therapies that target mutations or are particularly effective for molecularly defined subgroups, and therefore the MLL group looks for patient specific clonality or clonal patterns in CCUS and MDS.

One aim is to divide CCUS into subgroups according to the risk of developing MDS, as seen in figure to the right. This is done by the following methods:

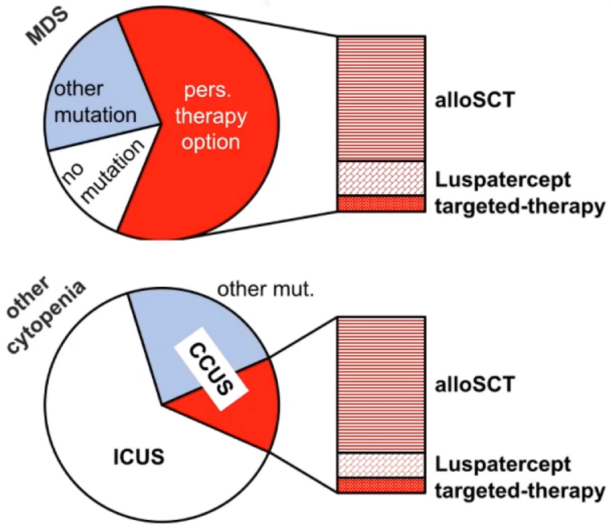


- Morphology and cytogenetic analysis according to WHO gold standard.
- Sequencing on NovaSeq after NextFlex library preparation and hybrid capture of a 41 gene panel.
- Pisces and pindel (BaseSpace, ILMN) analysis using a sensitivity of 3%.
- Variant classification by combining databases and in silico predictions (Hutter et.al, ASH 2019).

Naturally, in the 576 patients the median age for Idiopathic Cytopenia of Uncertain Significance (ICUS) diagnosis was 66 years, for CCUS 74 years and for MDS 75 years. More mutations were



found in MDS than CCUS patient's. ICUS is defined by absence of mutations.



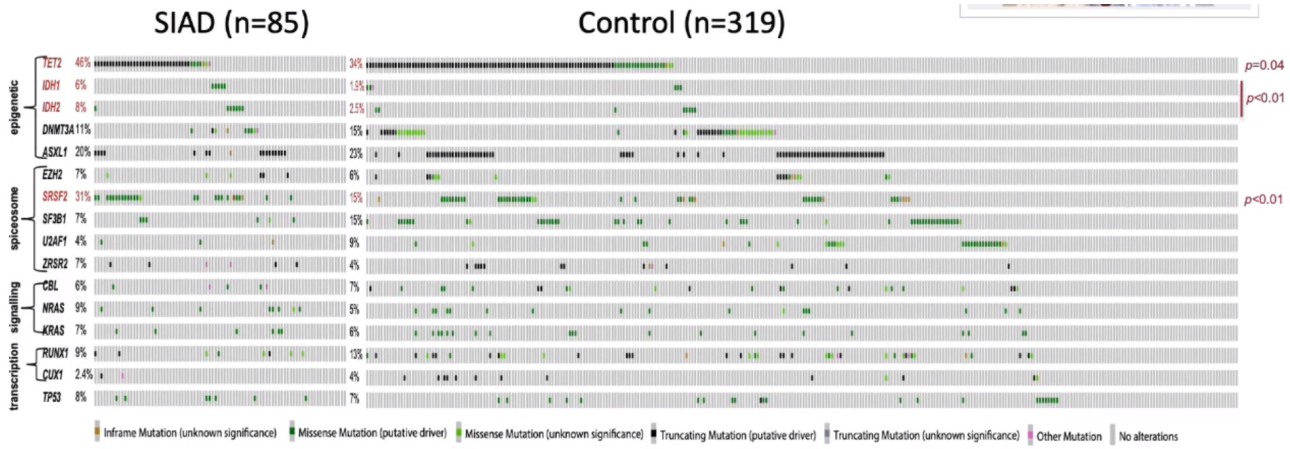
The diagram shows CCUS patients can have up to 4 mutations, but MDS patients can have more than 5 mutations. Further the International Working Group for the Prognosis of MDS (IWG-PM) suggests SF3B1 mutated patients are a special MDS subgroup.

The conclusion is that more and more subgroups will be defined based on specific mutations. The group further concluded that for ⅔ of MDS patient's and ⅓ of CCUS patient's mutations can be identified that allow personalized or risk-adapted treatment. They further suggest that molecular markers should be recognized as presumptive evidence of MDS and allow the

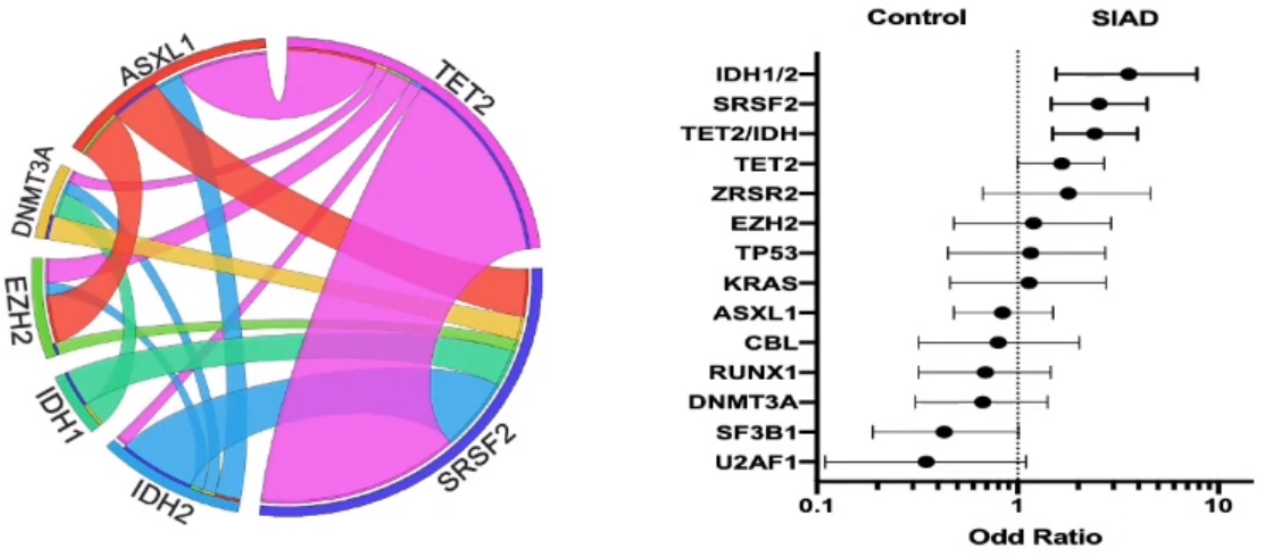
diagnosis to be based on three cornerstones:morphology, cytogenetics and molecular genetics.
I disagree with calling this approach personalized or risk-adapted treatment, as the identified treatment in many cases is HSCT (see figure to the left), and I don't know of any mutation, which suggests a HSCT.
It is well known among MDS doctor experts that if you give a sample for morphological analysis to two different pathologists, then there is a significant change you get to different results. I therefore would like to see the morphology element improved by e.g. using machine learning algorithms in place of human pathologists. This would also solve the problem of pathologists being overworked. Progress in this type of machine learning has been reported in the litterature, read e.g. here.

Do certain mutations indicate SIAD in MDS or CMML?

Some MDS patients experience systems similar to autoimmune diseases. This study by Dr. Lin-Pierre Zhao from Université de Paris looked at the molecular landscape of MDS or CMML with or without systemic inflammatory and autoimmune diseases titled "MDS/CMML with *TET2* or *IDH* mutation Are Associated with Systemic Inflammatory and Autoimmune Diseases (SIAD) and T Cell Dysregulation". This is a single center retrospective study of patients from the years 2012-2017. 404 patients with MDS or CMML were enrolled, and of these 85 had SIAD and 319 did not. However, only 77 of the 85 SIAD patients had bone marrow tests, and only 277 of the non-SIAD group had bone marrow tests. So not all participants were classified according to MDS subtype (MDS-SLD, MDS-MLD, MDS-RS or MDS-EB) and there were not patients from all MDS subtypes. Also the inclusion criteria were WHO 2016, which are newer than the time of first patient enrolment, so apparently they were re-classified according to WHO 2016. Hence I am a bit suspicious about the patient data.
The mutational landscape of all 404 enrolled patients is shown here (I know this is unreadable):



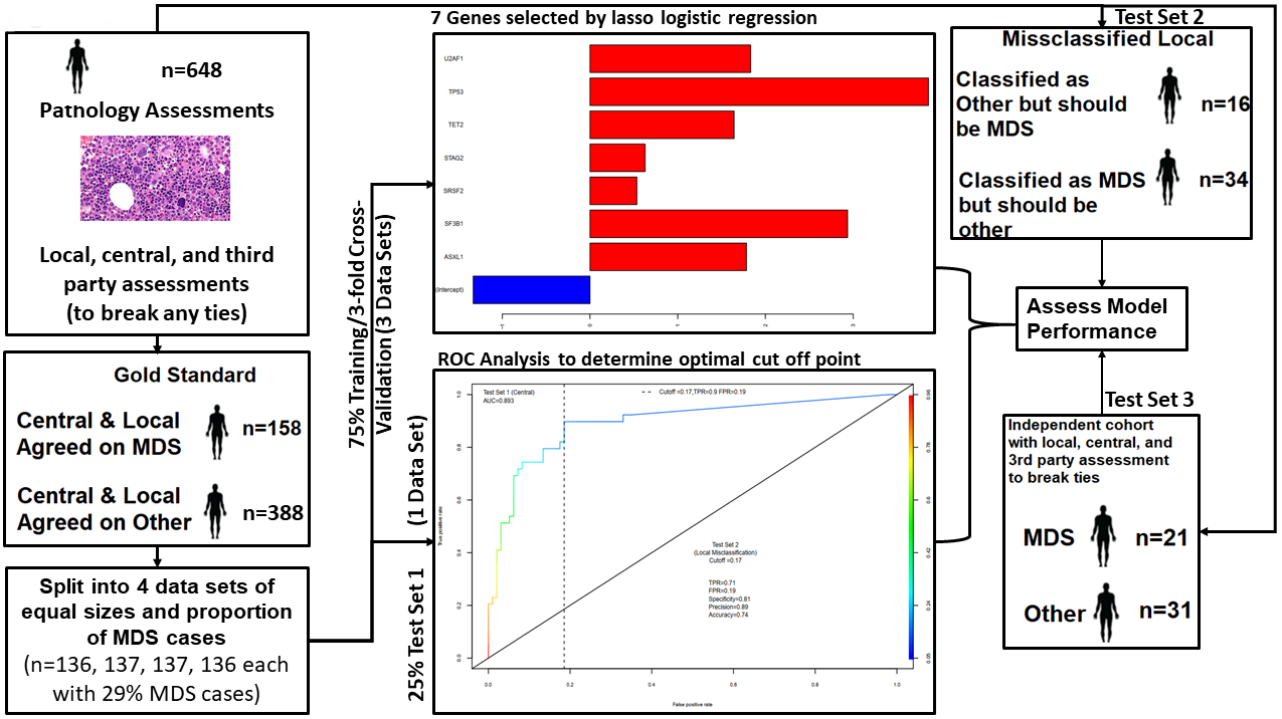
The NGS was done with a panel of 80 genes, but only the following are included in the above picture: TET2 (46%/34%), IDH1 (6%/2%), IDH2 (8%/3%), DNMT3A (11%/15%), ASXL1 (20%/23%), EZH2 (7%/6%), SRSF2 (31%/15%), SF3B1 (7%/15%), U2AF1 (4%/9%), ZRSR2 (7%/4%), CBL (6%/7%), NRAS (9%/5%), KRAS (7%/6%), RUNX1 (9%/13%), CUX1 (2%/4%), og TP53 (8%/7%). Based on this, the presenter claims SAID is associated with IDH1, IDH2, SRSF2 and TET2. However, it is not exactly clear what the basis for this is. I think the claim is based on the following graph.



To me the right graph indicates that U2AF1 and SF3B1 are mostly in the non-SIAD group, while as the authors state the IDH1, IDH2, SRSF2 and TET2 is mostly in the SIAD group. The left graph shows which mutations occur in pairs. I tend to interpret the left graph as follows: If there is a TET2 mutation, then 50% of the time there is also a SRSF2 mutation. Since not all patients had bone marrow tests I believe the NGS was performed on peripheral blood samples.
The authors conclude that further studies are needed to determine the exact role of IDH1, IDH2, TET2 and SRSF2 mutations in the origin of SIAD.

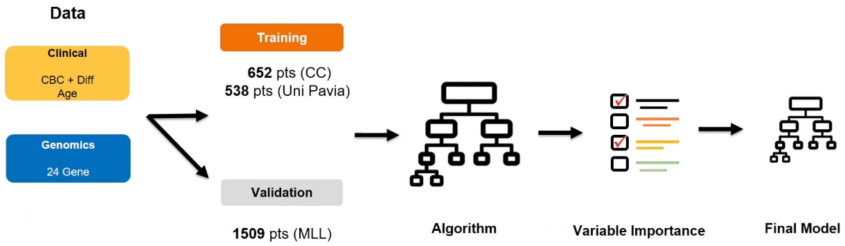
Targeted sequencing to reduce pathologic misclassification of MDS

J.B. Goll from The Emmes Company focused on pathologic misclassification in his presentation titled "Targeted Sequencing of 7 Genes Can Help Reduce Pathologic Misclassification of MDS" based on the prospective National Heart, Lung, and Blood Institute (NHLBI) National MDS Study (NCT02775383). The study involved two tests: one with a focus on disagreement between local and centralized pathology, and another with a new focus on a new group of patients. The genes most informative in predicting MDS were found to be: TP53, SF3B1, USAF1, ASXL1, TET2, STAG2 and SRSF2. The model reclassified 24 patients as MDS out of 50 where there was disagreement between central and local pathologists. The authors claim the gene based classifier resolved 74% of disagreements between central and local pathologists, and was 83% accurate in a prospective cohort of patients. However, I must admit that I don't really understand how the gene information is used prospectively, but the analysis is summarized in the following figure from the abstract:

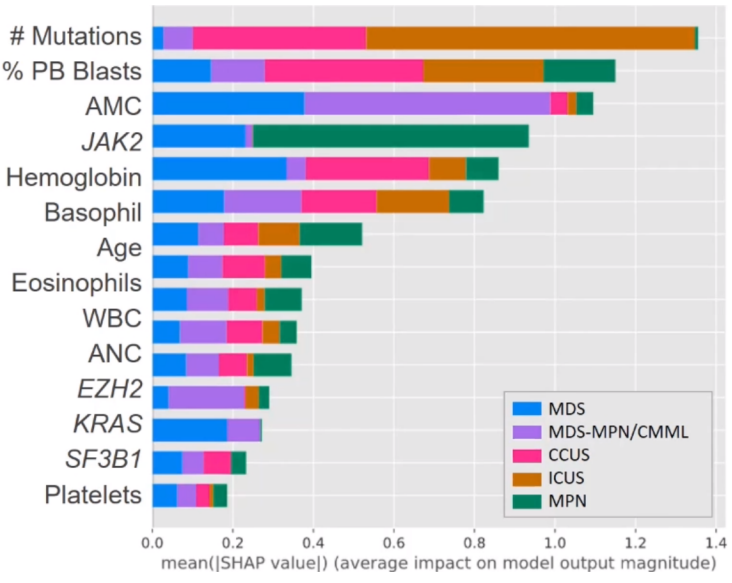


A Tool to Improve Diagnostic Accuracy in MDS

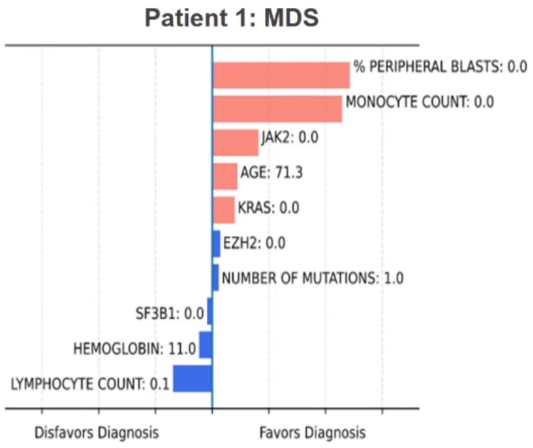
The final presentation in this clinical studies session was given by Nathan Radakovish from the Cleveland Clinic and titled "A Personalized Clinical-Decision Tool to Improve the Diagnostic Accuracy of Myelodysplastic Syndromes". The aim of this study according to the presentation was to develop a machine learning based method for differential diagnosis of myeloid malignancies independent of bone marrow biopsy data, according to the submitted abstract to develop a clinical model that uses mutational data and peripheral blood counts to distinguish MDS from other malignancies. The hope is this will reduce inter-observer variation in tissue evaluation, e.g. evaluation of dysplasia.



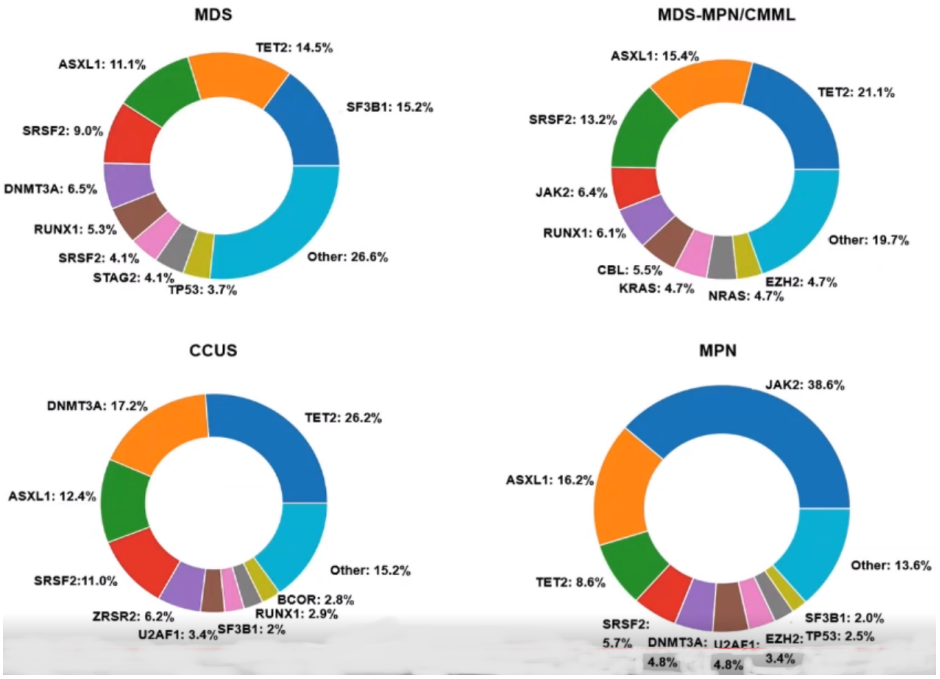
The model development used data from almost 3000 patients with MDS, Myeloproliferative neoplasms (MPN), MDS-MPN/CMML, CCUS or ICUS for whom blood count at time of diagnosis was available and NGS could be performed using a 24 gene panel. The model development process is shown in



the above figure. The result of the training was the important variables for each disease shown in the following graph:
So the number of mutations are important for CCUS and ICUS, while the presence of JAK2 mutation is important for MDS and MPN.



For each patient to be diagnosed the machine learning (ML) model shows for each variable in the model if that variable favours or disfavours a given diagnosis and to what extent, as seen for an MDS patient here:
Here MDS diagnosis is favoured by no peripheral blood blasts, the absence of JAK2 and KRAS mutations, age and monocyte count, but disfavoured by hemoglobin value and lymphocyte count. Thus the model doesn't give a diagnosis, but only indicates whether the available information favours or disfavours a particular diagnosis. It is still up to the hematologist to give the diagnosis!
The diagram to the left shows the mutations present in the different groups of patients. Many mutations, such as TET2 or ASXL1 are present in all disease groups. However, JAK2 is only present in MPN and MDS-MPN/CMML
I think it is positive that machine learning is being used to improve the difficult diagnosis of MDS, but I don't think we are ready to abandon dysplasia quite yet. Hence it will be important to develop ML tools that also improve the consistency of determination of dysplasia in both peripheral blood smears and bone marrow blood smears. Some patients look forward to not needing bone marrow biopsies.



Translational Molecular Diagnostics in Hematology

Already two days before the official opening of the ASH Annual Meeting there was a scientific workshop featuring 13 presentations divided into three parts: 1) overcoming challenges in delivering diagnostic genomics in hematology, 2) novel diagnostic genomic tools and technologies, and 3) how should we interpret what we find?

Implementation Challenges of Targeted Sequencing Panels

The opening talk was presented by Dr. Catherine Cargo from Leeds, UK titled "Implementation Challenges of Targeted Sequencing Panels" during which she stated, that the benefits of targeted sequencing panels is they allow sequencing of multiple genes in a large number of patients, is quick and affordable plus requirer manageable analysis and data storage. The challenges are

which genes to sequence, how many patients to sequence per run, and which library preparation / instrumentation to use. The genes are selected by the panels used, and there are two relevant panels: The myeloid / T-lymphoid panel covering 28 genes, or the B-lymphoid panel covering 33 genes (also known as the Sanger panel for high grade lymphomas), and then sequencing 48 samples per run using Fluidigm library preparation and MiSeq. Alternatively one could use the Pan HaemOnc Panel covering 233 genes including copy number detection, and also sequencing 48 samples per run using Twist library preparation and NextSeq. The later panel contains both clinically actionable genes and research genes, and hence - in my view - the data could be more useful for e.g. retrospective studies of subgroups of patients. It is important to store both raw data and analysis reports for future use. HMDS - Haematological Malignancy Diagnostic Services - does that and provides a web-based tool for clinicians to access the data. Dr. Cargo attempted to demonstrate the web interface, but the text on those slides was almost unreadable.
It should be noted that both England - soon UK - and Denmark have opted to implement national centers to manage the data created by next generation sequencing whether panel, WGS, or more elaborate sequencing. This relieves the individual researcher from this mundane task and in my view also ensures the security around access to these highly sensitive patient data. In England that is the NHS Genomic Medicine Service, which is a network genomic laboratory hubs to provide consistent and equitable care to patients, work to common standards,deliver a single national directory and facilitate research.
Dr. Cargo concluded that to be successful a service, such as provided by HMDS, needs sufficient manpower resources both for laboratory and IT, robust reporting and analysis infrastructure, and collaboration at the local, national and international levels.

Implementation Challenges of Whole Genome Sequencing

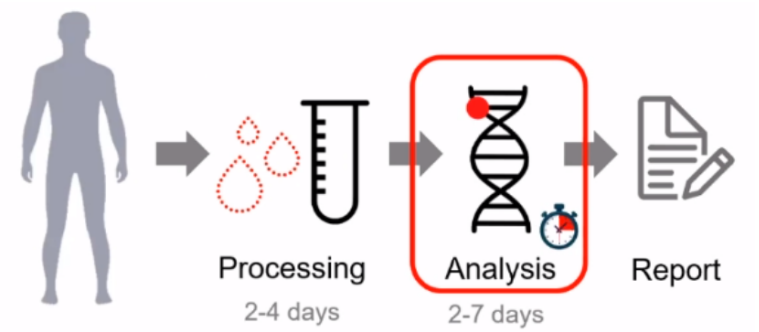
Dr. Torsten Haferlach from the Munich Leukemia Laboratory followed up with a presentation titled “Implementation Challenges of Whole Genome Sequencing” stating that WGS was beneficial in pediatric diseases, family diseases, rare diseases and cancer. He further stated that WGS involve three steps:

1 Library preparation
    1.a decide between enzymatic or mechanic fragmentation
    1.b PCR-free or with amplification
    1.c chose between single indexing or dual indexing
    1.d select quantification method
    1.e decide on amount of automation
2 Sequencing
    2.a decide on sequencing depth
    2.b decide on inclusion of control samples
3 Data analysis
    3.a infrastructure and data analysis
    3.b choose optimal tool for each analysis

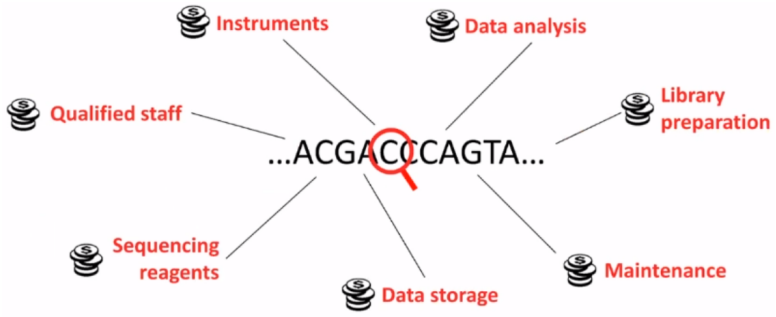
A key challenge is the choice of control sample, which involves noise reduction and identification of artifacts, as well as differentiation between germline and somatic variants. This is the base for variant calling. Another challenge is the depth of coverage as well as sensitivity.

In the clinical setting turnaround time from sampling to report availability

is also a challenge. In some situations a week is not an acceptable turnaround time. Computing power and parallelization may need to be looked at.
The final challenge with WGS is cost; one of which is staff for specialized tasks in both laboratories and IT.

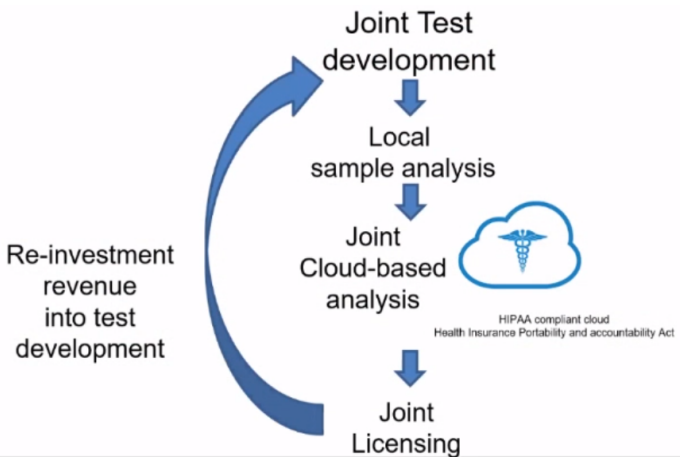


I believe none of these costs are trivial and all seven are important for the result, i.e. better molecular diagnostics in hematology.



Diagnostic Genomics form a Global Perspective

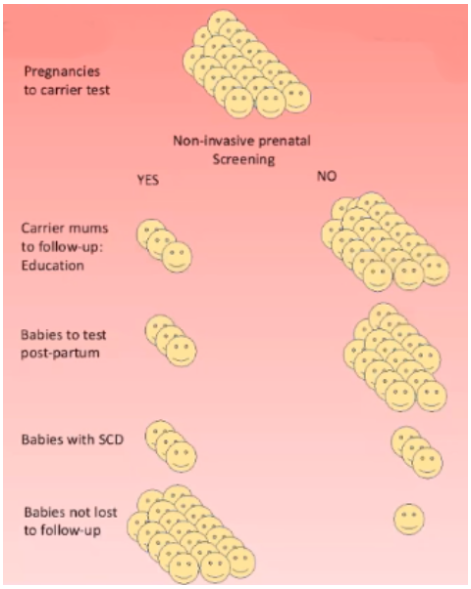
Dr. Anna Schuh from University of Oxford gave the third presentation which in the outline was titled “Diagnostic Genomics from a Global Perspective”, but her actual presentation had the title “SEREN: A social enterprise to build capacity in DNA-based diagnostics that improves survival of children with blood diseases in sub-Saharan Africa” which was the winner of the 2020 University of Oxford Vice-chancellor Award for innovation.



The problem addressed is that in sub-Saharan Africa many blood diseases that can be easily cured or controlled with affordable therapies are not diagnosed due lack of affordable easy-to-use diagnostic technologies, a functioning supply train, trained clinical and laboratory staff, and quality control. The solution is a social enterprise as shown in the functional diagram to the right. The first disease to be tackled was sickle-cell disease needing a genetic diagnosis. So far the following solutions have been provided:

1. Detection of mutations and/or deletions that affect the HBA1, HBA2 or HBB genes and can be performed on whole blood samples or dried blood spots with sequencing on a MinION instrument using a simple-as-possible library preparation instrument and a cloud- based bioinformatics pipeline with fast turnaround time, and a cost of 11 USD for a comprehensive haemoglobinopathy test.
2. Screening maternal plasma for affected babies and focus follow up and education on 100 out of 10.000 babies instead of 10.000 using a method for non-invasive prenatal diagnosis that allows precise quantification of fetal allele fraction without need for paternal genotype for detecting autosomal dominant or recessive diseases characterized by single base pair changes where mum carries exactly the same mutation as fetus.

3 Nanopore sequencing to diagnose leukemia by using amplicon and WGS libraries combined on a single flow cell to generate information regarding IgHV status, TP53 mutations and del(17p) in patients suspected to have leukemia.



4 WORK-IN-PROGRESS Liquid biopsy to detect tumor DNA from lymphomas or screen for lymphomas.

Dr. Schuh concluded that SEREN brings third generation sequencing, cloud-based analysis, and cell free circulating DNA sampling to sub-Saharan Africa using innovative business and licensing models in collaborative health research effort that addresses the needs of the patients in the area.

This was the end of the first part of this Scientific Workshop with a focus on overcoming challenges in delivering diagnostic genomics in hematology.

Whole Genome Optical Mapping

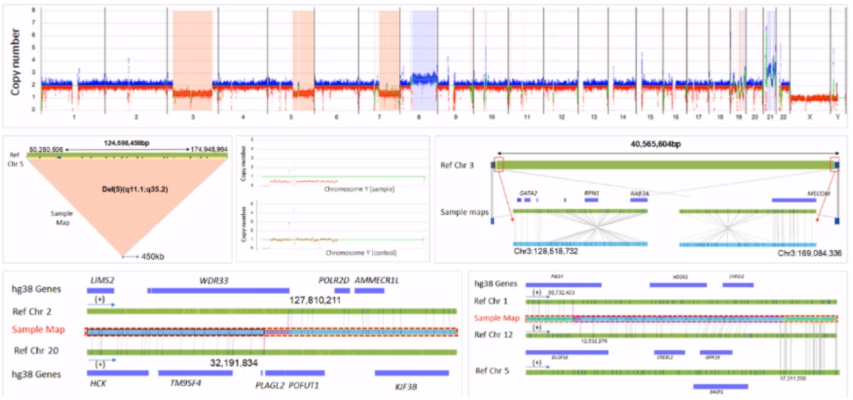
The first presentation in the 2nd part of this Scientific Workshop was by Dr. Rashmi Kanagal-Shamanna from the MDS Anderson ~~Cancer~~ Center in Houston with the title “Whole Genome Optical Mapping” and the subtitle “A Novel Molecular Diagnostic Tool for Comprehensive Assessment of Structural Chromosomal Variations in Hematological Malignancies”. This was a presentation on the absolute forefront of analysis of sequencing, and hence I am not certain I got all the details right.

The method involve the following steps:

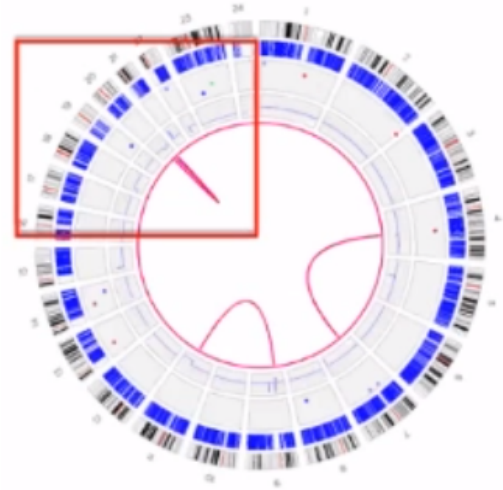
1 Isolation of high molecular weight DNA. About 1 million cells with a concentration of more than 36 nanograms per deciliter is needed, and it may be obtained from frozen cell pellets from the bone marrow, bone marrow mononuclear cells preserved in MNSO (a technology before cryo-perservation), or left over specimen from clinical flow cytometry.

2 Direct label and stain.

3 Linerize DNA.

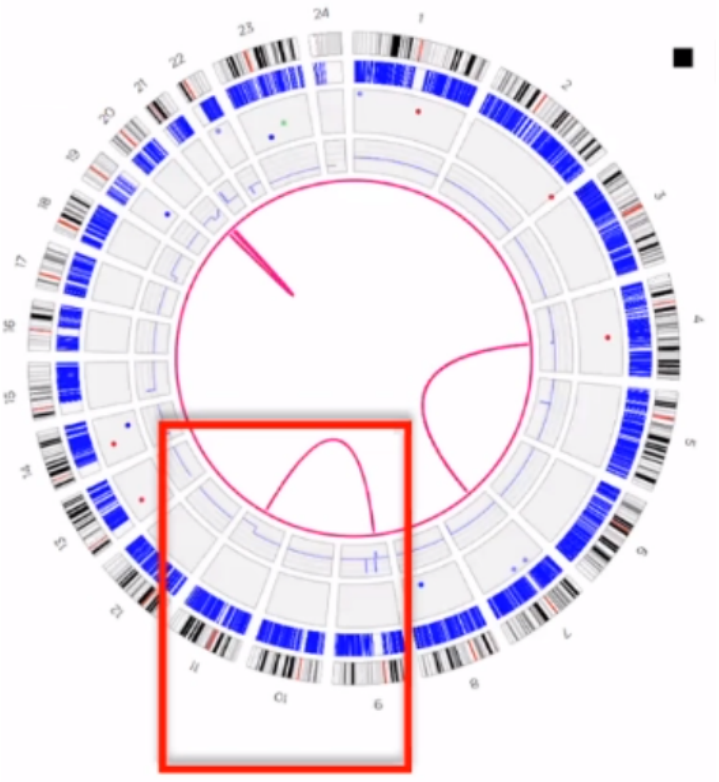


4 Imaging.

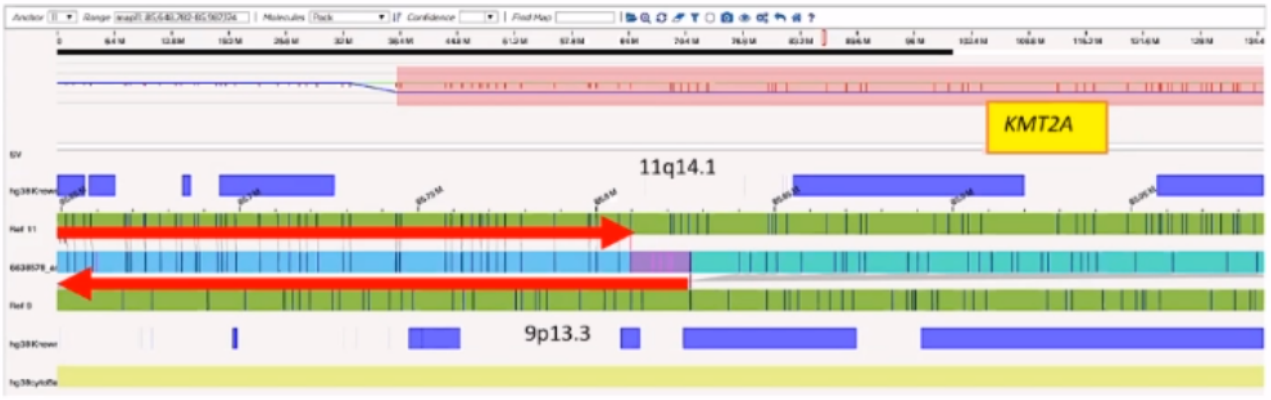


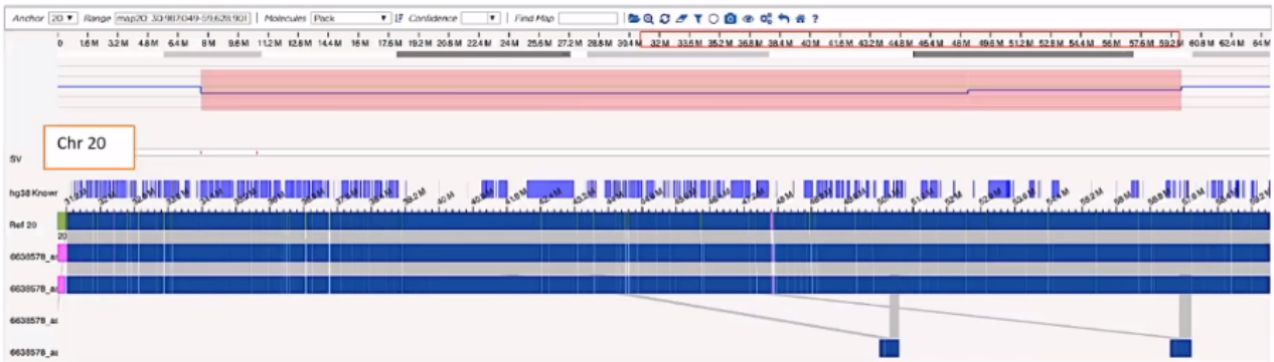
The results can be difficult to read, on a linear display, as shown here of all structural variants in MDS / AML. Therefore scientists have come up with CIRCUS (click link to watch a video about this tool), which represent each chromosome as a circle.

Here is a CIRCUS plot of the same data, and from here one can zoom in on an area of interest, e.g. the red rectangle.This is data from an MDS patient with 2% blasts and mutations t(9;11) and del(20q).



Below are shown linear plots of the two area highlighted in the above CIRCOS-plots:





If you are not familiar with these types of plots, then I can recommend the introduction to genomics at National Human Genome Research Institute.

Dr. Kanagal-Shamanna concludes that the approach presented provides a single platform assay for accurate identification of all types of structural variants and voids the need for multiple

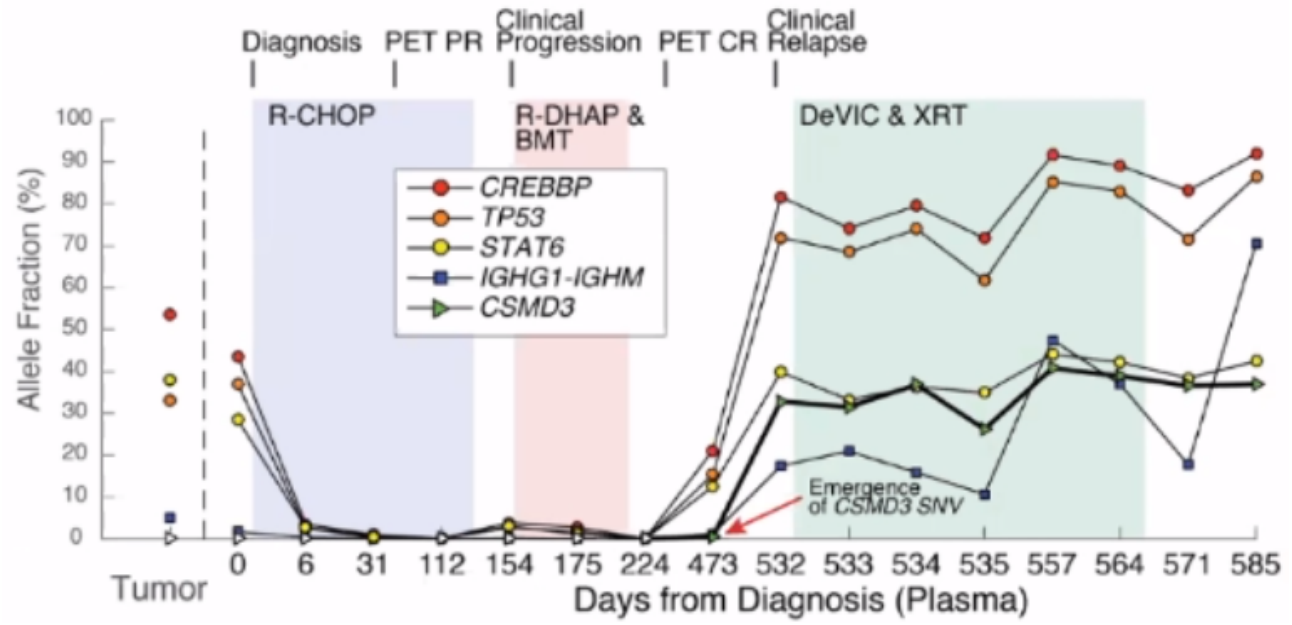
confirmatory tests, provides faster turnaround time and is less expensive. However it does not detect copy neutral loss of heterozygosity (cnLOH).

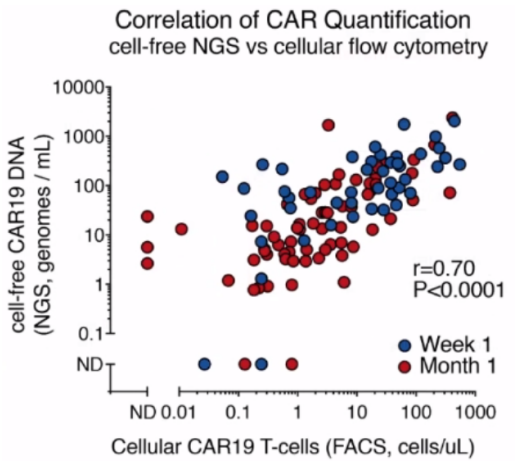
CAR (Chimeric Antigen Receptor) T-cell Dynamics from Cell Free Desoxyribonucleic Acid (DNA) Sequencing

The second presentation in this section of the Scientific Workshop was by Dr. David M Kurtz from Stanford University Medical Center on “CAR T-cell dynamics from cell free DNA sequencing” the basic idea of which is to use circulating tumor DNA (ctDNA) from liquid biopsies as biomarkers in lymphomas of patients treated with CAR-T therapy. The methodology is called Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) for Lymphomas, and its development involve the following steps:

1 Sequencing panel design. Create CAPP-Seq panel for population analysis covering 334 genes to find single nucleotide variants.
2 Find cancer mutations in tissue and in plasma.
3 Track mutations in plasma.

The following diagram show the tracking of several mutations in ctDNA samples from taken since diagnosis:





Treatment is noted at the top starting with R-CHOP followed by R-DHAP. Notice the new mutation appearing around day 473 since diagnosis. Encouraged by the above they designed a new panel for CAT T-cell therapy in large B-cell lymphoma. This showed correlation between cell-free NGS of circulating blood DNA and cellular flow cytometry,

Dr. Kurtz has discussed the ideas presented here in a video on OncLive in 2018. The conclusion here two years later are

1 ctDNA can be used in CAR19 T-cell therapy for tumor monitoring, CAR19 T-cell monitoring and T Cell Receptor (TCR) monitoring.
2 CAR-cfDNA quantification correlates with CAR flow quantification.
3 ctDNA and CAR-cfDNA have prognostic significance.

Genome-wide Copy Number Calling from Targeted Sequencing Platforms

The third presentation in this second part focused on Novel Diagnostic Genomic Tools and Technologies in this Scientific Workshop prior to the ASH Annual Meeting was given by Dr. John F. Markham from Peter MacCallum Cancer Centre in Australia and titled “Genome-wide Copy Number Calling from Targeted Sequencing Platforms”. Dr. Markham started by comparing results on different vendors' equipment. For genome wide copy numbers the off-target percentages are all over the map. Dr. Markham showed results from different vendors' equipment, but unfortunately the figures were unreadable, the learning from this presentation was minor.

Assay	% Off Target
Illumina TSO 500 (hyb capture)	25%
Agilent SureSelect (hyb capture)	60%
QIASEQ (UMI-based SPE)	5%
Twist Bioscience (hyb capture)	70%

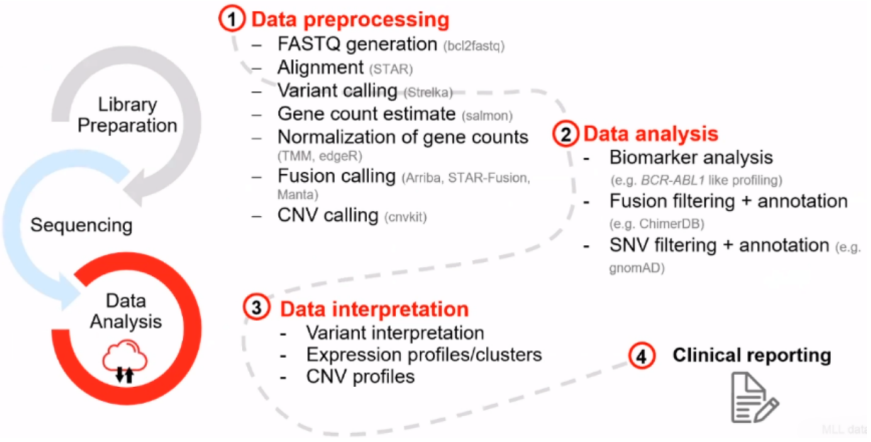
Dr. Markham recommended

- alignment whole genome reference and use of an alignment upstream of unique molecular identifier processing.
- use of suitably chosen, historical, technically matched samples to provide reference mean and variance estimates.
- use metrics to confirm that the samples really are technically matched.
- estimate copy number at suitably chosen resolutions and plot at multiple scales.
- visualize the data before reporting

Not being an NGS expert the only conclusion I draw from this presentation is that whole genome copy number estimation is more an art than a tool ready for the clinic at this moment.

Whole Transcriptome Sequencing in Diagnostic Genomics

The fourth presentation was given by Dr. Manja Meggendorfer from the Munich Leukemia Laboratory (MLL) with a presentation titled “Whole Transcriptome Sequencing in Diagnostic Genomics”. Dr. Meggendorfer started with discussing two different choices of library preparation protocols: Ribosomal Ribonucleic Acid (rRNA) depletion or Poly(A) selection. rRNA depletion is a critical method in transcriptomics that allows for efficient detection of functionally relevant coding as well as non-coding transcripts through removal of highly abundant **r**RNA species. Read more about the difference between the two approaches here. According to Dr. Meggendorfer rRNA depletion gives slightly better results in novel fusion detection, while Poly(A) selection is slightly more accurate in gene expression profiling.

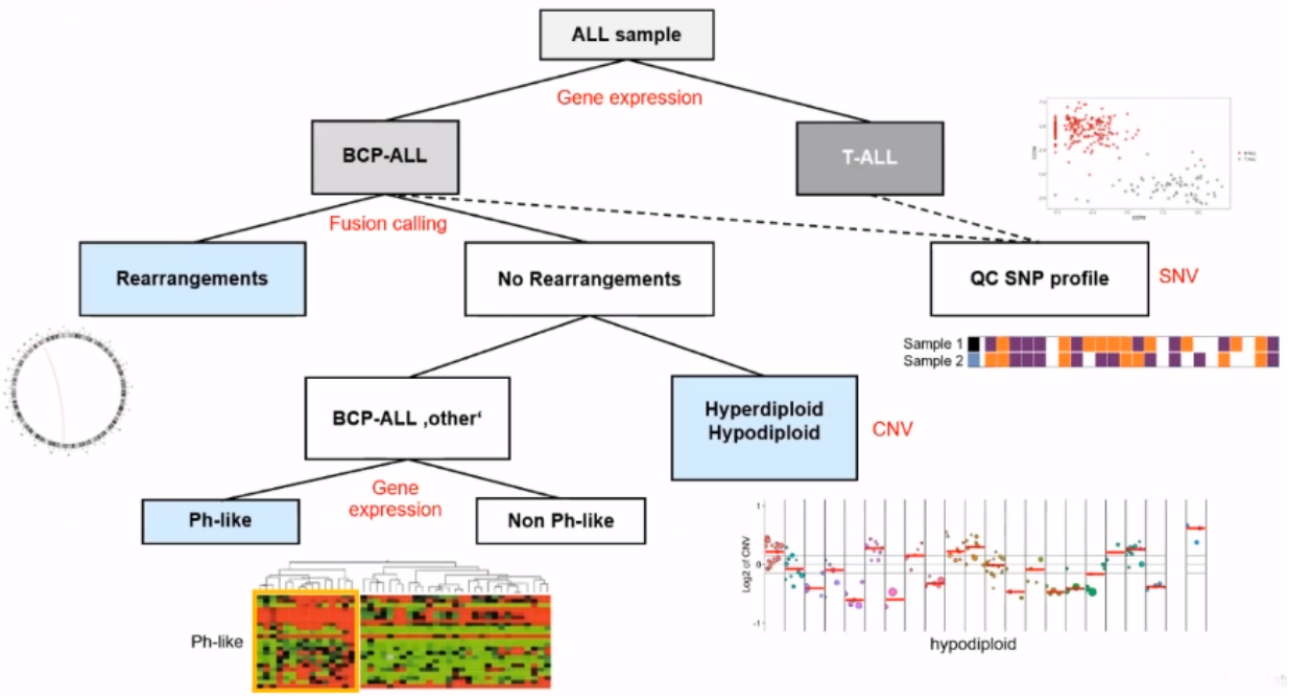


The workflow at MLL involves four steps: 1) Data preprocessing - basically focus on what you want, 2) Data analysis - biomarkers and annotation, 3) Data interpretation - variants, expression and profiles, and finally - for the benefit of the patients - clinical reporting. Here is as an example the results for an Acute Lymphocytic Leukemia (ALL) patient:

Naturally this display just gives you an idea of the output for two items: Single-Nucleotide Polymorphism Quality Control (QC SNP) profile and Non Philadelphia Chromosome (Ph)-like.

Dr. Meggendorfer also showed data for an AML patient with the RUNX1 mutation 46,XY, del(17)(q11q22) with a novel fusion gene due to deletion involving MS12 transcriptional regulator and TLK2 serine/threonine kinase, which could suggest a kinase inhibitor treatment.
Dr. Meggendorfer stated, to be successful with whole transcriptome sequencing one have to realise that

- choice of adequate library prep is a most

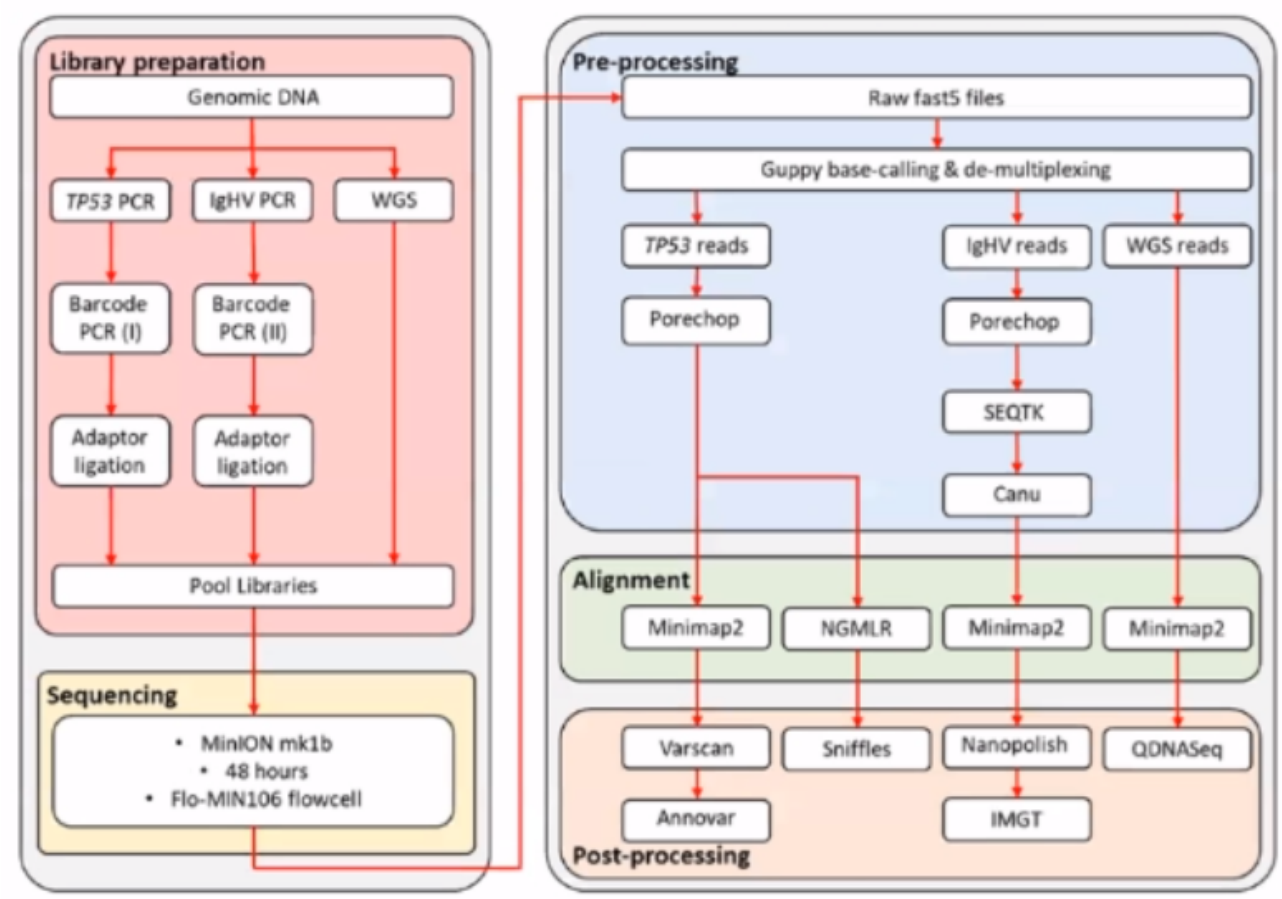


- wet lab work is time consuming
- proper analysis of the data requires bioinformatics expertise
- many analyses are possible based one data set
- several leukemia and lymphoma entities can be classified
- comprehensive nature of assay allows identification of novel markers, and
- treatment targets

I wonder how long before we will see this type of analysis used at time of diagnosis in centers, such as the Clinic for Blood Diseases at Rigshospitalet in Copenhagen. A center which is already deeply into translational research.

Unlocking Chronic Lymphocytic Leukemia with a Benchtop Sequencer

The final presentation in this section of this Scientific Workshop was by Dr. Niamh Appleby from Oxford Molecular Diagnostics Centre, and the title of her presentation was “Unlocking Chronic Lymphocytic Leukemia with a Benchtop Sequencer: The Potential Future Role of Nanopore Sequencing in Molecular Diagnostics”.
The aim of this project is to in a single run using a Nanopore-based Chronic Lymphocytic Leukemia (CLL) specific screening assay detect TP53 disruption mutations and del(17,p13.2) as well as IgHV status. And the patient group include early asymptomatic CLL patients, frontline clinical trial patients, and TP53 disrupted relapsed / refractory patients.
The methodology involve the five steps 1) Library preparation, 2) Sequencing, 3) Pre-processing, 4) Alignment, and 5) Post-processing as seen in this diagram:



Dr. Appleby concluded that nanopore long-read sequencing permits coverage of full exonic regions of TP53, full length IgHV genes, and detection of clinically relevant del(17p) as well as detection of genomic complexity.
The question that immediately comes to mind is whether it is worthwhile to develop technology such as this aimed at patients you already suspect or know have CLL or one should aim at technology covering more leukemia? or both leukemia, lymphoma and MDS?

This was the end of the second part of this Scientific Workshop with a focus on novel diagnostic genomic tools and technologies.

What are Germline and Somatic Variants and What do they tell us?

The first presentation in the 3rd part of this Scientific Workshop was by Dr. Catherine E. Cottrel from Nationwide Children's Hospital titled "An Overview of Germline and Somatic Variant Curation". A germline mutation is a change in the body's reproductive cells, which are passed on from parents to offspring, and a somatic mutation is a change in any other cells in the body. Dr. Cottrel started her presentation by showing this gene cloud:

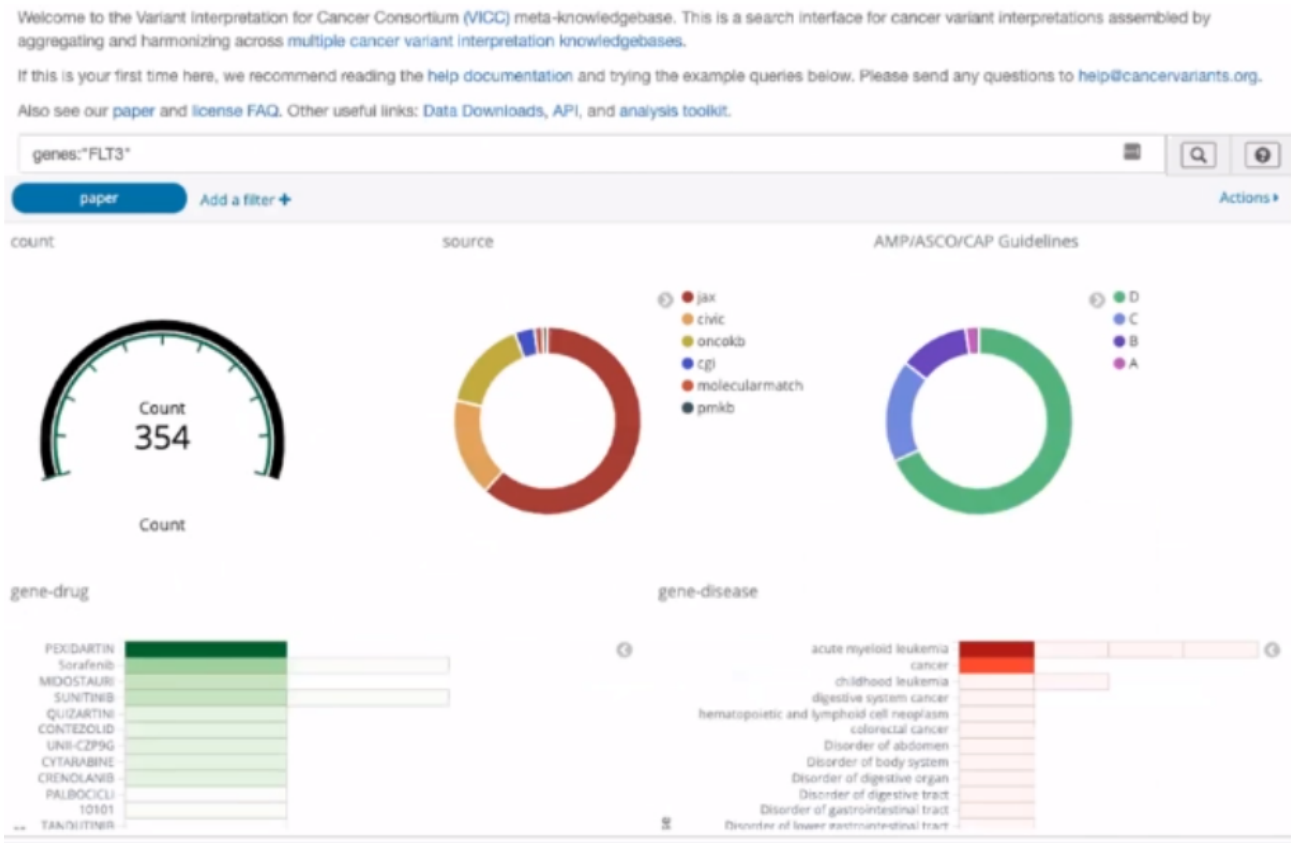
A variant is a single change in a single base in a gene, where one base in the DNA string is changed to another base. These changes can be polymorphous, uncertain, pathogenic or therapeutic, and Dr. Cottrel pointed to "Standards and Guidelines for the Interpretation of Sequence Variants" (link to guidelines) by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, and

pointed out the evidence found here. Dr. Cottrel also pointed to another guideline specific to cancer. Both of these guidelines continue to be improved, and in my view is a good starting point for discovering what next generation sequencing already can contribute with.

Next Dr. Simone Feurstein presented worked examples related to germline curation. But started to point out the germline mutations in table 17 of the 2016 WHO Classification of Myeloid Malignancies , before discussing rules related to mutations in RUNX1.

Many Free Resources on the Internet

Similarly Dr. Kalannin Krysiak presented worked examples related to somatic curation, and provided a link to a spreadsheet with a collection of resources, and highlighting her involvement in two of these: CIViC and VICC. Dr. Krysiak discussed 2 variants: 1) STAT3 p.Y640F in Large Granular Lymphocytic Leukemia, and 2) FLT3 c.1773_1793dup in Acute Myeloid Leukemia while pointing out the evidence in the different databases, and showing this output from search.cancervariants.org:



Interpreting output such as this requires dedication and training. However, I find it very fascinating that many resources such as this one are freely available on the internet. But unfortunately time is limited for us all.

ASH and Precision Medicine

The final presentation of the workshop was given by Dr. Alex Wagner on “ASH Precision Medicine Working Group: Integrating Variant Interpretations”. The classifications of variants agreed to by this working group is freely available on the ASH website under Research, Gene-tabel. It is worth

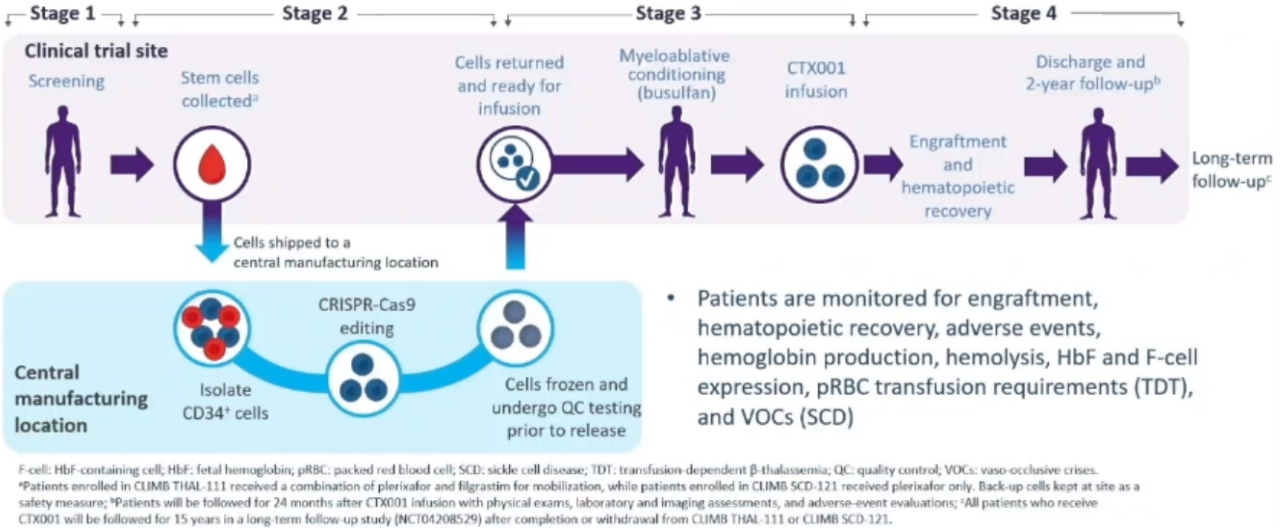
mentioning, that this ASH working group has a close collaboration with the Munich Leukemia Laboratory.

ASH Sunday Plenary Session

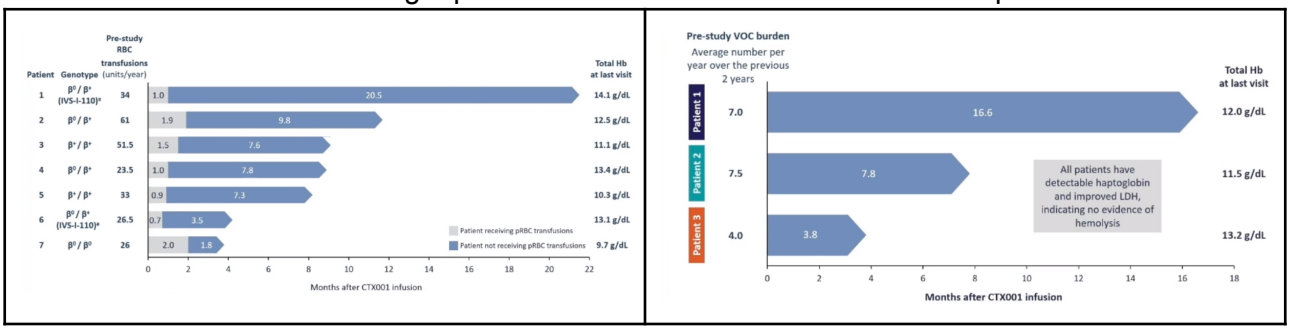
The Sunday plenary session featured 6 presentation:

1. "Loss of LBK1/STK11 Facilitates Leukemic Progression of the Myeloproliferative Neoplasms" was the abstract title, which in the presentation given by Dr. Christian Marinacciowas was re-titled to "STK11/LBK1 is a Tumor Suppressor in the Leukemic Progression of Myeloproliferative Neoplasms", such polycythemia vera (PV), essential thrombocythemia (ET), myelofibrosis (MF) or myelodysplastic syndromes (MDS) progressing to acute myeloid leukemia. This was a mouse study with some comparison with data observed in actual patients.
2. "Effects of Tranexamic Acid Prophylaxis on Bleeding Outcomes in Hematologic Malignancy: The a-TREAT Trial" was the abstract title, which in the presentation given by Dr. Terry B. Gernsheimer was re-titled to "The American Trial Using Tranexamic Acid in Thrombocytopenia (A-TREAT)" and by the introducer to "Prevention of Bleeding in Patients with Hematologic Malignancy and Thrombocytopenia". The conclusion of the trial was that tranexamic acid did not reduce bleeding events.
3. "BCL10 Gain-of-Function Mutations Aberrantly Induce Canonical and Non-Canonical NF-κB Activation and Resistance to Ibrutinib in ABC-DLBCL" was the abstract title, which was also the title of Dr. Min Xia's presentation.
4. "Safety and Efficacy of CTX001 in Patients with Transfusion-Dependent β-Thalassemia and Sickle Cell Disease: Early Results from the Climb THAL-111 and Climb SCD-121 Studies of Autologous CRISPR-CAS9-Modified CD34+ Hematopoietic Stem and Progenitor Cells" presented by Dr. Haydar Frangoul. *The data from the two trials was very preliminary and based on small numbers of patients. Nonetheless was the transfusion independence spectacular at least to me. CTX001 appears to be a game changer in these groups of patients, who so far have lived with blood transfusions. I cannot stop speculating if a similar potentially curable treatment approach could be possible for other hematologic malignancies which originates from errors in the stem cells.*
5. "Divergent Levels of CD112 and INKA1 Define a Subset of Human Hematopoietic Stem Cells That Resists Regenerative Stress to Preserve Stemness" was the abstract title, and also the title of Dr. Kirsten B. Kaufmann's presentation. Stemness refers to the ability of stem cells to replicate themselves and their potential to differentiate into other types of cells. So stemness is a combination of 'self-renewal' and 'differentiation' capabilities. This study was on mise.
6. "Poor Treatment Outcomes of Young (<60 Years) African American Patients Diagnosed with Acute Myeloid Leukemia (Alliance)" was the abstract title and also the title of Dr. Bhavana Bhatnagar's presentation. Not surprisingly the African American group had poorer outcomes than a comparable group of White Americans. In Denmark we see such differences between the well educated (with a university degree) and people without anything beyond elementary school in all interactions with the health care system.

I will not go into details about these plenary presentations except for the CTX001 study, which at the time of data cut-off had enrolled 7 β-Thalassemia and 3 Sickle Cell Disease patients. The first of the following figures show the treatment procedure:



and below the results of treating 7 β-Thalassemia and 3 Sickle Cell Disease patients:



Very nice to see the CRISPR-CAS9 technology finding its way to the clinic in the same year the inventors of the technology received the Nobel Prize in Medicine for their discovery of this technology. This is the ultimate personalized treatment in hematology.

Conclusion

This ASH 2020 Virtual provided spectacular news about a CRISPR-CAS9 based treatment of β-Thalassemia and Sickle Cell Disease. I think that development will find its way to other hematological diseases, such as MDS, PNH and related diseases in the next 5 years.
Within MDS there is an ongoing trial to expand the use of Luspatercept to more low risk MDS patients, than the initial approval for the RS group. Also there are many promising drugs for higher risk MDS in different stages of clinical trials. I will especially be following APR-246 and the TIM-3 inhibitors the next little while.

This years virtual format of the ASH conference allowed you to go back to watch most presentations several times over a 3 week period. I found this much better than cramping all into some very busy 3 days.

Niels Jensen
MDS patient and member of LyLe
Slangerup, 2021-03-29

PS: I did also attend both the MDS Foundation and AA & MDS International Foundation Satellite Symposia. If you need info about these please contact me at niels.jensen@mds-and-you.info .