ASH2024

Saturday Myeloid Oncogenesis

Jie Sun
Multi refractory leukemia disease→sequence, sc-Target-seq→TFG-ROS1 fusion を発見
TFG-ROS1 has leukemogenic activity and Kinase activity of ROS1 is important.
Sensitive to ALK/ROS1 inhibitor (Ceritinib/Crizotinib)
Confirmed the clinical effect of Ceritinib/Crizotinib in the patient → CR in 13 months.
＊難治性白血病を sequence して rare fusion を発見し、それが白血病誘導能を持つことを発見し、阻害剤を使って患者さんを治療したら治った、という話。今後こういうことが普通にできる時代が来るか？

Mark Althoff
MCL1 promotes Fatty Acid b oxidation in therapy resistant AML
MCL1 interacts with transcriptional repressor IRF2BP2 in primary Ven/Aza resistant AML.
IRF2BP2 is aberrantly sequestered in the cytoplasm in Ven/Aza resistant cells.
IRF2BP2 has BH3-like domain, which mediates the interaction with MCL1.
S63845: BH3 specific MCL1 inhibitor.
BH3-targeted MCL1 inhibition → IRF2BP back to nucleus
Ven/Aza resistant LSC: Fatty Acid metabolism up
IRF2BP2 controls transcription of the fatty acid metabolism gene ACSL1
ACSL1 inhibitor suppresses Ven/Aza resistant AML
ACSL1 inhibitor has no effect on normal CD34+ cells.
＊MCL1 の non-canonical な function の話。Ven/Aza resistant は Monocytic AML に多いので、monocytic AML で大切な経路なのかもしれない。

Dongxu Jiang
TET2 regulates stability of mismatch repair protein MSH6 and helps maintain genomic stability independent of its dioxygenase function
Mutation Burden increases in myeloid neoplasms associated with TET2 mutations.
Loss of TET2 increases sensitivity to Mutagen and promotes leukemogenesis in vivo.
TET2 interacts with MSH6 and colocalizes in the nucleus.
TET2 is essential for MSH6 protein stability.
Loss of TET2 impairs the mismatch repair in Genomic DNA.
＊TET2 の non canonical function の話。ASXL1 も何かの質量分析で MSH6 と結合して

いた気がする。

Jonas Fullin：The Pathogenesis of therapy related Myeloid neoplasms from TP53-mutant clonal hematopoiesis
Trp53-R245W を mono or biallelic に持つ mouse を用いて解析
Trp53 hets promote clonal expansion
Biallelic Trp53 promotes genomic instability and promotes leukemic transformation.
Mdm2 overexpression phenocopies biallelic Trp53 mutations.
＊R245W は monoallelic でも clonal expansion を誘導するが、白血化には biallelic mutation が必要という話。

Maria Latacz：Recurrent ASXL1 mutations promote myeloid malignancies through unleashed condensation
ASXL1 WT has IDR at C-terminus but cancer-associated mutants lose it.
Hypothesis: ASXL1 WT forms condensates and mutant lose it.
Actually, mutants but not WT form condensates.
Positive charge at N-terminus drives condensation of ASXL1.
ASXL1 truncation condensates promote H2A deubiquitination by enriching BAP1.
The negative charge in the frequently deleted region controls ASXL1 condensate formation.
＊普通は IDR が condensation には重要なことが多いが、ASXL1 の場合は N 末端の positive charge が condensates の formation に重要で、WT では mutation hotspot 周辺の negative charge がこれを抑制しているが、mutation でこの部位の negative charge が失われると condensates を作るようになるという衝撃の内容。パラスペックルとの関連も含めて検証の必要あり。

Saturday Chemical Biology and Experimental Therapeutics

Amit K Jaiswal：IGFBP3 inhibitor with potent antileukemic activity
IGFBP3 is frequently overexpressed in cancers including MLL-AF4 B ALL.
IGFBP3 is dispensable for normal hematopoiesis but essential in MLL-AF4-driven ALL.
Development of IGFBP3-RNA interaction inhibitor using TR-FRET
IGFBP3-GFP + biotin labeled RNA oligo saturated with terbium labeled Streptavidin.
1st Screening >190,000 compounds→ Cell based counter screen using IGFBP3 KO cells
→ I3IN-002 as the best hit
I3IN-002 alters gene expression similar to IGF2BP3 KO; down of BCL2, HOXA9, CDK6.
More effective in MLL-r B-ALL compared to non MLL-r ALL and AML

I3IN-002 binds to IGFBP3 and inhibits RNA binding ability

＊Screening で hit した標的を対象に、dCas13-NanoBiT などを使って、こういうことができないか？逆に、そこまでいけないとやっている意味が無いとも言える。

Markus Meyerhofer：p300/Crebbp are context dependent functional repressors of Interferon Genes, offering therapeutic promise in AML

p300 is essential for AML survival

p300 inhibition by (1)KAT domain inhibition (KATi) (2)bromodomain inhibitor, (3) degrader → All compounds suppress the key AML maintenance genes

Only KATi induced Interferon Stimulated Genes (ISGs) specifically in monocytic AML

P300 interacts with cBAF complex (ARID1A, SMARCA4) and transcription factors (RUNX1, IRF8, PU.1 MEF2D, and IRF2BP2.

KATi and IFNa co-treatment induced exponential increase of the ISG response and a highly synergistic induction of apoptosis in human and mouse monocytic AML cell lines.

p300 maintains a repressive environment for ISGs in conjunction with the cBAF complex in monocytic AML.

＊p300 を KATi で抑制した時の表現型は SETDB1 KO とほぼ同じ→ 同じ complex か？KATi+INFa 併用両方で強い治療効果→SETDB1 阻害剤や STUB1 阻害剤でも同じことが出来るか？

Majd Al-Hamaly: *In vivo* zebrafish drug screen uncovers novel insights into LSC biology

Rag2-Myc-induced T-ALL zebrafish model: LSC frequency 1:10

Screen >700 FDA-approved drugs using > 2500 zebrafish for the effect on self-renewal

Secondary screen in human T-ALL→4 compounds that decreases the LSC frequency

Limiting dilution→ Amiloride, an inhibitor of the Sodium Hydrogen Exachanger-1 (NHE1)

NHE1 is responsible for the anticancer effects of Amiloride.

NHE1 knockdown inhibits LSC function and induces G1 cell cycle arrest.

NHE1 downregulates stem cell genes, Myc-target KRAS, Hedgehog, Notch signalings.

NHE1 knockdown inhibits mitochondrial function, downregulates OXPHOS.

→ reprograms metabolism in T-ALL

＊Zebrafish Screen の成功例

Saturday Emerging Tools, Techniques and AI in hematology

Vera Binder Blaser：ALL-R: ananchoring score to predict relapse in ALL using zebrafish patient-derived xenograft model

Zebrafish xenotransplantation model + time-lapse confocal microscopy
Leukemic cells from patients with early relapse (ER) are more confined in their movement than those from patients without relapse (NR).
An anchoring score (ALL-R score): velocity, max distance traveled, displacement, confinement ration, straight line speed.
ALL-R scores were higher in ER patients than NR patinets.
ER leukemic cells highly express adhesion molecules such as CXCR4.
CellChat suggests that ER leukemic cells are likely interact with ER monocytes through MIF-CD74/CXCR4 and CD99/PILRA.
＊早期再発の ALL 患者の白血病細胞は、じっとしていてあまり動かない傾向があるとのこと。何を意味するのか？興味深い。

Hnna Duan：Genomic subtypes of AML define sensitivity to NK cells
CD14+ blast cells are more sensitive to NK cells than CD34+ blast cells
ELN risk score does not determine the sensitivity of CD34+ cells to NK cells
BCOR and RAS mutated cells are more sensitive to NK cells
Co-culture→ Sensitive AML cells induce NK cell activation
＊Monocytic AML は NK 細胞に感受性が高い傾向があるらしい。

Li Li: TP53 mutations within T cells induce T cell exhaustion and functional impairment in TP53 mutant AML
TP53 mutations can be detected in CD4+CD8+ T cells and NK cells
TP53 mutated CD123 CAR-T cells: enhanced survival but more exhausted
Mutant p53 impairs CAR-T cell anti-AML cytotoxicity
p53-Y220C reactivator in TP53 mutant T cells enhances antitumor activity
＊TP53 変異があると、CAR-T 細胞の生存、増殖は向上するが、その分 exhaust してしまい、抗 AML 効果は減弱してしまう。

Sunday Therapeutic Gene Editing

Paulo Rio: Generation of a Stealth FLT3 and KIT functional variants
Introduce mutation in FLT3 and KIT to evade antibody recognition, without changing function in human HSCs by Base Editor.
Edited cells are resistant to CAR-T.
FLT3 edited HSPCs display normal engraftment in mice.
FLT3 CAR-T eliminates AML while sparing edited HSPCs.

KIT epitope editing can be multiplexed with BCL11a editing.
Different mAb regiments improved co-selection of edited cells.
Epitope Editing confers protection from a high affinity KIT-Ab.
＊ヒト造血幹細胞で自由自在に editing している。うちでもできるはず！

Julia Skokowa
Gene therapy of ELANE-associated mutations.
Autosomal dominant ELANE mutations in all exons and two introns.
ELANE encodes a neutrophil elastase.
ELANE is a paralog of PRTN3 and is dispensable for neutrophil differentiation.
(ELANE mutation perturb it: Dominant negative?)
Knockout of ELANE recovers neutrophil differentiation.
NETs formation is normal without ELANE
＊変異型 ELANE は好中球分化を抑制するが、ELANE KO は抑制しない。Dominant negative or Gain of function 効果か？

<u>Sunday Plenary Session</u>

Rachel Rau: Blinatumomab added to chemotherapy improves survival of pediatric B-ALL
Standard risk groups: Randomized trial
Blinatumomab significantly improves disease free survival
Blinatumomab reduces leukemic burden in marrow but not in CNS
Low incidence of blinatumomab specific toxicities
But higher rates of subsequent sepsis and catheter related infections
＊小児 B-ALL は Farber 博士が最初に化学療法を行った腫瘍で、化学療法が最も良く効く腫瘍として有名だが、ここ数年は予後の大きな改善がみられず化学療法による治療成績向上は限界に達した感があった。今回腫瘍免疫の力を使った CD19-CD3 BiTE (Blinatumomab)と化学療法を組み合わせることで、さらなる治療効果 up に成功したという話。CD19-CD3 BiTE は今後 B-ALL 治療の主役になりそうな感じ。ただ、CNS 病変には効果が無さそう。

Sen Zhang：PF4 regulates HSC aging
Aging disrupts the megakaryocyte niche
PF4 expression is downregulated in old megakaryocytes
Young PF4-KO mice: exhibit premature HSC aging(myeloid skew, increased DNA damage…)
Administration of recombinant PF4 to old mice reduces DNA damage of HSCs

PF4 administration improves aged HSC function in vivo
LDLR and CXCR3 are the PF4 receptor in HSCs.
CXCR3/LDLR dKO mice exhibit accelerated HSC aging.
PF4 also regulates human HSCs and rejuvenates Aged HSCs.
＊PF4 を分泌する血小板が HSC の niche として働くという以前の研究結果に基づき、PF4 分泌と老化の関連を調べた研究。PF4 投与で老化造血幹細胞を若返らせることができそう。

Shan Liu:Ketogenic Diet enhances CarT cell antitumor function via b-Hydroxybutyrate (BHB)
It has been shown that antibiotics treatment reduces CART effect.
It has been shown that Gut microbiome affects CART function.
→ Food can affect CART function.
DLBCL tumors (A20) were transplanted to Balb/c mice and they were fed with five representative diets: ketogenic, high-fiber, high-fat, high-protein, Western(high cholesterol) diet, and then infused anti-CD19 CAR-T cells (CART19).
The ketogenic diet potentiates antitumor CART19 effect.
BHB promotes CART proliferation in vitro and enhances CART function in an immunocompetent murine model
BHB improves human CART function in a human xenograft model
BHB increases peripheral IFN.
BHB enters TCA cycle preferentially over glucose and enhances the OXPHOS in CART cells.
BHB concentration in patients correlates with CART cell expansion
Clinical trial now open
＊Rizap も採用している ketogenic diet(糖質制限をして脂肪を燃えやすくする diet)で CART 活性が up するとのこと！

David J Kuter: The effect of BTKi on ITP
Rilzabrutinib: Oral Reversible BTK inhibitor
No side effect to inhibit platelet aggregation
Rilzabrutinib improved physical fatigue, even among nondurable platelet responders
No severe side effect
＊BTK inhibitor が血小板数を増やすことは少し前から知られていたが、同時に血小板の機能を落としてしまうため ITP 治療に使えなかった.Rilzabrutinib は血小板機能抑制がほとんどない BTK inhibitor で、今回 ITP への有効性が RCT で証明された。他の自己免疫疾患への効果も期待される。

Sunday Myeloid Oncogenesis

Xueer Wang：Mitochondrial RNA methylation by METTL17
LSCs depend more on OXPHOS than HSCs
RNA binding proteins + Mitochondrial proteins: mtRBPs
METTL17 is associated with poor prognosis of AML
METTL17 KO suppresses OXPHOS and reduces LSC frequency
METTL17 promotes leukemogenesis of MA9.3-NRAS and MOLM13
METTL17 needs methyltransferase activity and mitochondrial localization to promote AML growth (MTS and Mtase domains are required).
CLIPseq: METTL17 directly catalyzes 12S rRNA m4C and m5C methylation
12S rRNA methylation maintains its stability and steady state level
METTL17 regulates retrograde mitochondrial nuclear communication: Reduced CoA, Citrate Succinate, H3K27ac down
Core gene: CCND3. METTL17 enhances CCND3 expression via H3K27ac
CCND3 can rescue the effect of METTL17 depletion
METTL17 siRNA + TLR9 ligand: AML など TLR9 発現細胞にのみ取り込まれる。
Phamacologically targeting METTL17 inhibits leukemogenesis
＊ミトコンドリア RNA 修飾に関与する分子を探索するため、RNA Binding Protein でかつミトコンドリアに存在する分子を探索し、その中で AML の予後不良や AML 細胞の増殖(DepMap)に関与するものとして METTL17 を同定。TLR9 と siRNA を組み合わせた創薬方法も興味深い。

Stefan Bjelosevic: Riboflavin Drives nucleotide biosynthesis and Iron sulfur metabolism to promote AML
Human plasma mimicking media→　Vitamin depletion screens
Riboflavin (Vitamin B2) is a leukemia dependency in NB4 and MOM13
Riboflavin Kinase (RFK) is a leukemia dependency in AML
Riboflavin loss→down of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Supplementation of FMN and FAD rescued the effect of Riboflavin depletion.
Riboflavin depletion induced downregulation of pyrimidine, causing nucleotide imbalance.
Depletion of multiple purine biosynthesis enzymes and uridine supplementation promoted cell growth in riboflavin deficient media.
Nucleotide imbalance was accompanied by mitochondrial disruption.
RFK depletion or depletion of exogenous riboflavin perturbs mitochondrial respiration and

sensitizes to BCL2 inhibition
Riboflavin loss also reduced iron sulfur cluster proteins
＊Vitamin B2 が AML の増殖に必須とのこと。メカニズムはいろいろだが、Pyrimidine の量が減って nucleotide のバランスが崩れてミトコンドリア活性が落ちるというのが面白い。Purine の量も減らしてバランスととってあげると、増殖が回復するらしい。

RNA binding protein RBMX maintains leukemia cell growth survival
RBMX is highly expressed in AML and control nascent transcript RNA
LC/MS: RBMX interacts and colocalizes with YTHDC1, the main nuclear m6A reader
RBMX and YTHDC1 are found within the same regions in a cell
YTHDC1 is reader is required for leukemogenesis
RBMX forms gel-like condensates that do not undergo LLPS
RBMX regulates biophysical properties of YTHDC1 mediated condensates
RBMX and YTHDC1 share targets at RNA and DNA levels.
Generation of the rapid RBMX depletion system (RBMX-FKBP) in THP1 cells
RBMX depletion induces rapid changes in gene expression, chromatin accessibility in 4 hours
YTHDC partially rescued the effect of RBMX depletion.
RBMX creates more fixed YTHDC1 condensates that sequester RNA pol II
＊YTHDC1 が RBMX と interact し、ゲル状の condensates を形成して RNA や gene expression 制御に関与するという話。パラスペックルとの関連は？

Jihye Yoon: Fetal differentiation programs afford a protective barrier to NUP98-fusion driven AML that dissipates shortly after birth.
NUP98 rearrangements (NUP98r) cause high risk AML primarily in early-to-mid childhood.
NUP98r is rare in adult AML, and never occurs before birth.
Generated iPSC that give rise to chimeric mice expressing diverse NUP98 fusions.
NUP98-HOXA9 expression; fetal or juvenile induction
NH inhibit repopulating ability only in fetal cells
Juvenile NH causes leukemia more efficiently than fetal induction
NH dramatically reprograms epigenome and transcriptome of fetal cells and drives erythroid differentiation.
NH did not alter hematopoietic differentiation in juvenile progenitors and only upregulated HOX genes.
＊NUP98 fusion は fetal liver 細胞は transform しないが、若いマウスの projenitor は transform するという話。かなり意外で retrovirus モデルではこうなるとは思えない。iPS モデル（詳細はわからなかった）だからか？大人（老齢）マウスはどうなのか？

Monday Molecular Pharmacology and Drug Resistance

Samantha Levin Furtney: REM-422 a small molecule MYB mRNA degrader
REM422 acts by promoting MYB poison exon inclusion to induce MYB mRNA degradation
Poison exon inclusion
REM-422 reduces MYB mRNA and protein levels and phenocopies MYB genetic KD in THP1 cells
Oral dosing of REM422 drives regression in AML CDX model
Oral dosing REM422 eradicates blast in AML PDX model and induces differentiation and apoptosis
＊MYB の転写の時に poison exon を読ませるようにする低分子化合物の話。作用機序が極めて興味深い。どうやってスクリーニングしたのか？RUNX でも同じようなことができないか？

Nour Naji：CC chemokine receptor like 2(CCRL2) is a potential therapeutic target in MDS and secondary AML
CCRL2 is upregulated and promotes growth in erythoroleukemia
Anti CCRL2 antibody drug conjugate ADC
CCRL2 ADC induces apoptosis and suppresses colony formation, leukemogenesis of TF1.
CCRL2 ADC shows selective cytotoxicity and induces apoptosis in TP53 mutated AML
No toxicity against normal hematopoietic cells
No toxicity to normal erythropoiesis
CCRL2 ADC treatment is safe in B6 mice
＊AEL の新しい治療標的。ADC はやはり強い。

Monday Myeloid Oncogenesis

Dimitrios Papaioannou: CRISPR/RfxCas13d screening identifies NFYC-AS1 lncRNA as a molecular vulnerability in AML
179 prognostic lncRNAs (1048 gRNAs) for dropout screening in KG-1, MOLM13, OCI-AML3 and THP1 cells.
24 essential lncRNAs in all 4 cell lines, 9 sutype-specific essential lncRNAs.
NFYC-AC1: important in monocytic AML with NPM1c or KMT2A rearrangements.
High NFYC-AC1 expression is associated with poor prognosis
NFYC-AC1 is enriched in nucleus , but not on chromain.
NFYC1 regulates chromatin conformation of the chr19p13.3
＊lncRNA 情報も増えてきた。少し出遅れたか…

Travis Fleming

Stem cell gene expression, which is associated with poor prognosis, is frequently driven by MECOM in AML

MECOM-dTag rapid degradation system

MECOM degradation causes AML differentiation and cell death

MECOM is a direct repressor of myeloid differentiation in AML

MECOM repressed genes and cisREs are conserved in primary AML

MECOM repress CEBPA cisRE

＊MECOM-degron でマウスモデルを作っても良いかも。