"Drug Response Profiling":
Test *Ex-vivo* Di Nuove Molecole Per Attuare Trattamenti Personalizzati Nelle Leucemie Acute Linfoblastiche Ad Alto Rischio
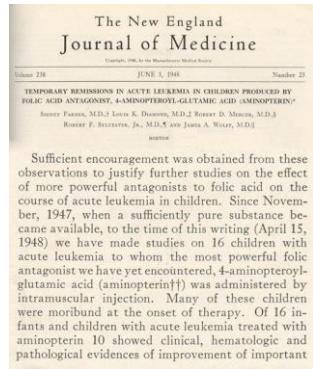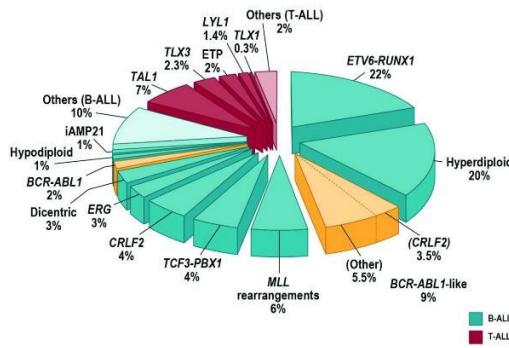
Luca Pagliaro, MD, PhD

Ematologia e CTMO
Translational Hematology and Chemogenomics (THEC)
Azienda Ospedaliero Universitaria di Parma

Disclosures of Luca Pagliaro

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

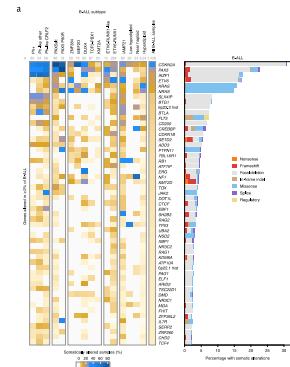# Acute Lymphoblastic Leukemia: Platform for Innovation

Chemotherapy

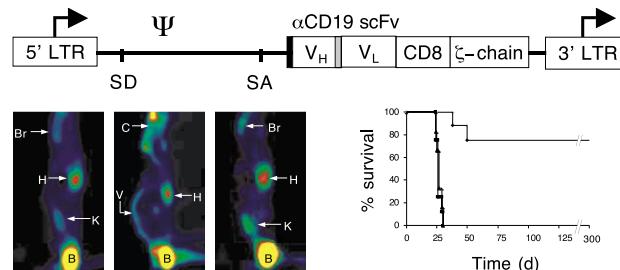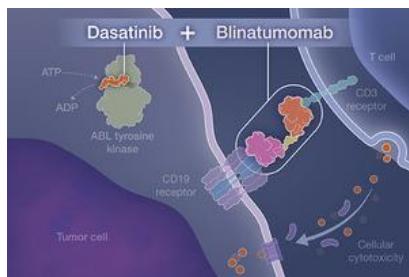**Clinical Significance of Cytogenetic Abnormalities**
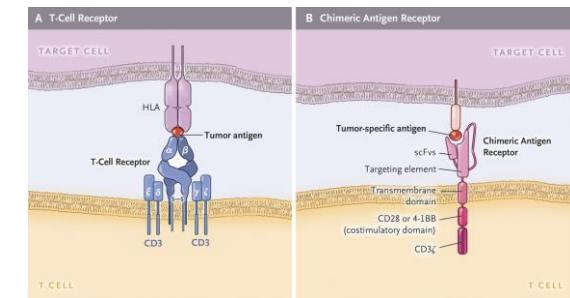
Gene Expression Profiling

Mutational Landscape

Farber S. N Engl J Med 1948; 238:787-79
Mullighan CG. Hematology Am Soc Educ Program 2012;389–96.
Armstrong SC. Nature Genetics volume 30, 41–47 (2002)
Brady SW et al. Nature Genetics volume 54, pages 1376–1389 (2022)
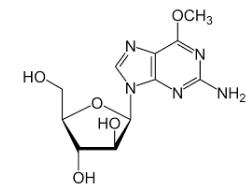
Acute Lymphoblastic Leukemia: Platform for Innovation
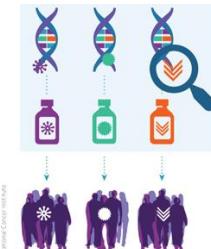

Bispecific T cell Engager

Adoptive T cell Therapy | CAR-T

Foà R, N Engl J Med 2020; 383:1613-1623
CH June, M Sadelain. N Engl J Med 2018;379:64-73.
Brentjens, RJ, Nature Medicine 9, 279–286 (2003)

Relapse/Refractory T-ALL: a Clinical Challenge

- A gap between the knowledge of the mechanism and pathways involved in leukemogenesis and the empirical nature of the first line therapies proposed to treat patients with T-ALL → But Why Does it Work?

- None of promising B-ALL approaches is registered for T-ALL.
  - Immunotherapy
  - Car-T cell therapy
  - Tyrosine kinase inhibitor

- The only registered drug for R/R is nelarabine (Antriance, EMEA/H/C/00752)

- Precision medicine tools (NGS) to patients with AL are often unrealistic at relapse.
  - NGS→ 2 weeks
  - PDX→ 2 months

- Most patients receiving genomic testing do not benefit from a genomic precision medicine strategy. NCI-MATCH trial
  - 38% actionable mutation
  - Of those 18% had a success to a relevant treatment (single arm)
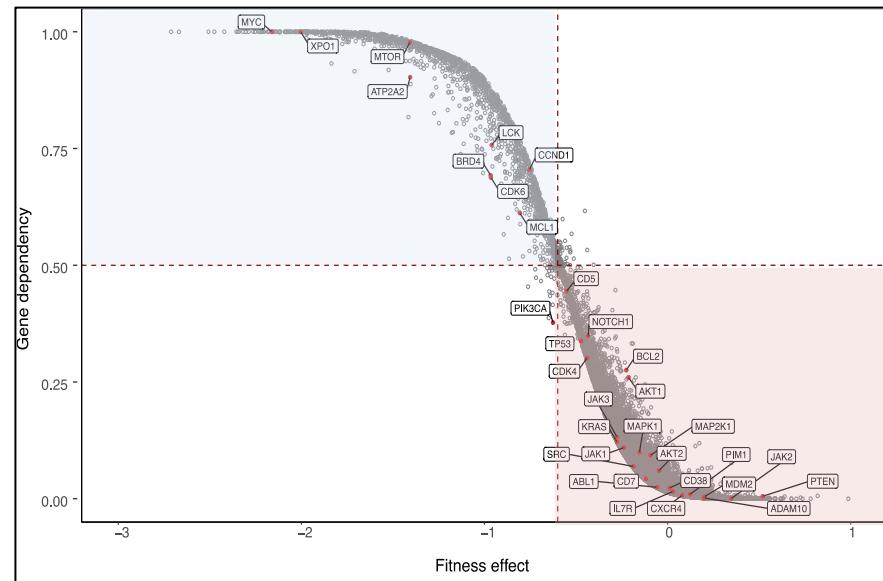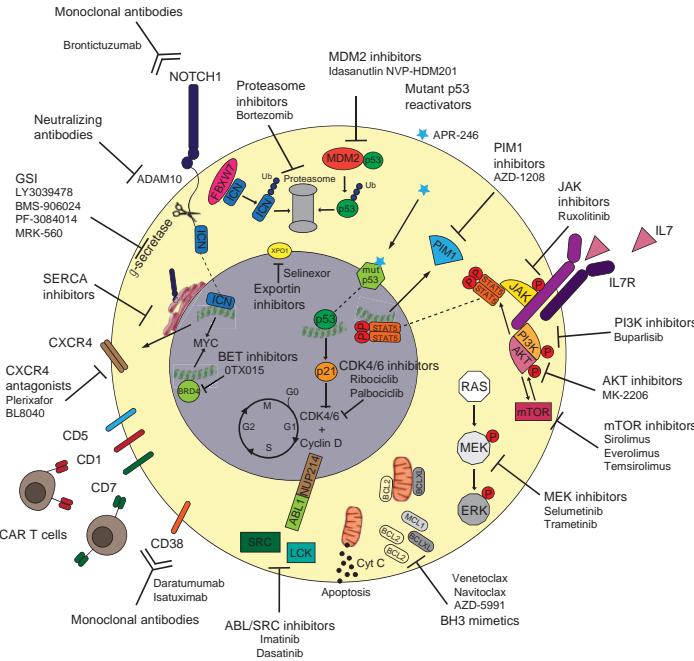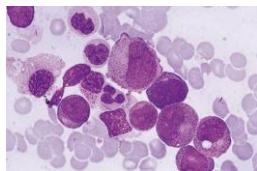 - ORR 2-38%
  - NCI-ComboMATCH

**Nelarabine
28, OCT 2005**

NCI-Match trial

*Semin Oncol 2016 43(1) 9-12
Jama oncol. 2016;2(6):717-718
Cancer Discov. 2021 Jan;11(1):6-7*

Target Therapies to Tackle T-ALL Vulnerabilities

Chemical Genomics: Elucidating Biological Systems with Small-Molecule Compounds

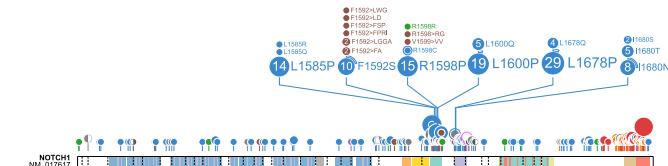**Phenotype of Interest**
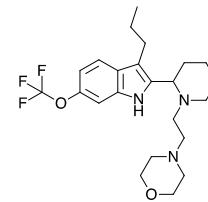
Forward
Hypothesis-generating

Reverse
Hypothesis-generating

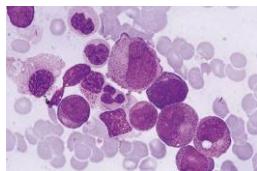**Gene/Protein of Interest**
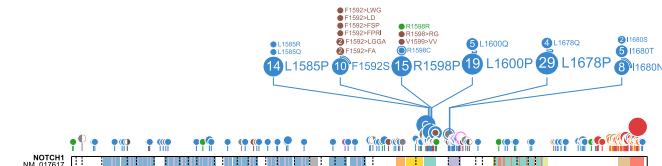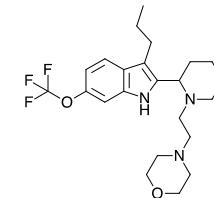
Small Molecule Library

Targeted Small Molecule

Chemical Genomics: Elucidating Biological Systems with Small-Molecule Compounds

Phenotype of Interest

**Forward
Hypothesis-generating**

**Reverse
Hypothesis-generating**
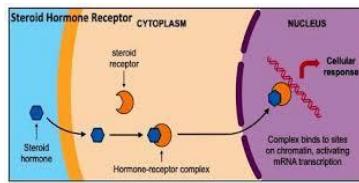
**Population Level
Chemogenomic Screen
New Drugs for Subset
of HR Leukemia**

Small Molecule Library

Gene/Protein of Interest

Targeted Small Molecule

Targeting Transcription Factors
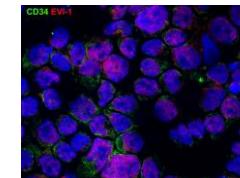
Nuclear Receptors
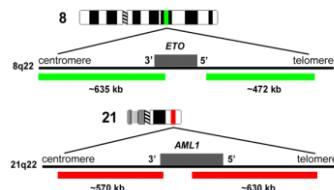
Latent Cytoplasmic Factor

Roti G et al., Cancer Cell 2013; Roti G et al. JEM 2018
Marchesini M & Gherli A., Cell Chemical Biology 2020
Pagliaro et al., Journal of Hematology & Oncology 2021
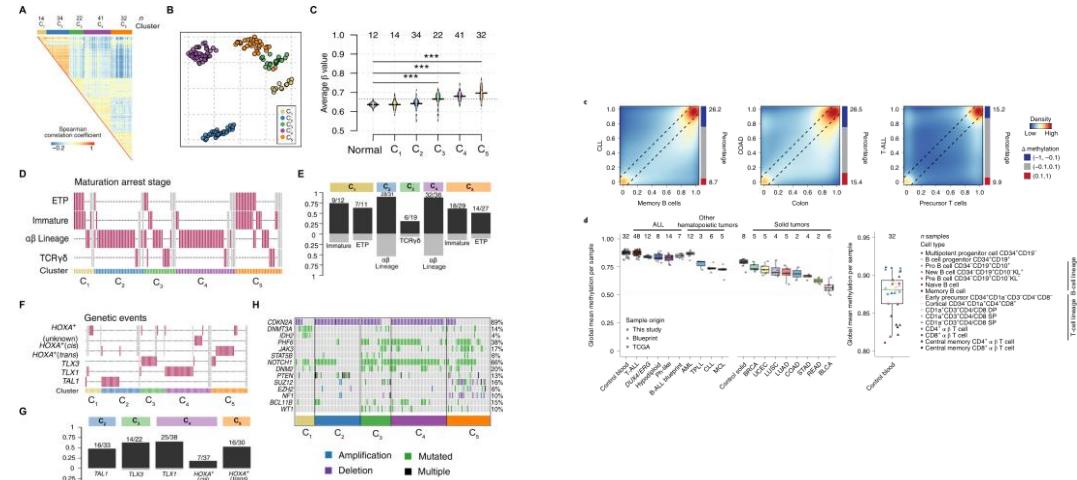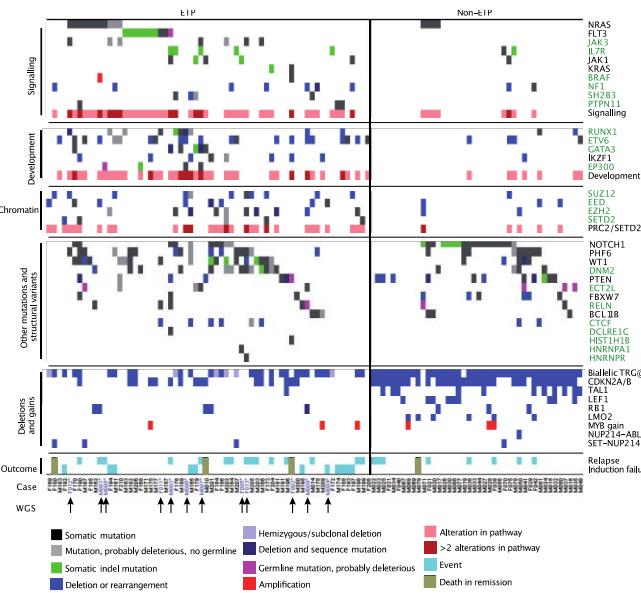
**Oncogenic Translocation
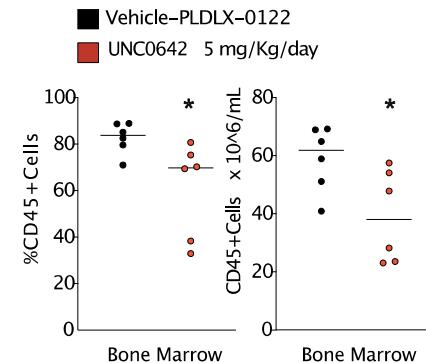Fusion Proteins**

Resident Nuclear Factor
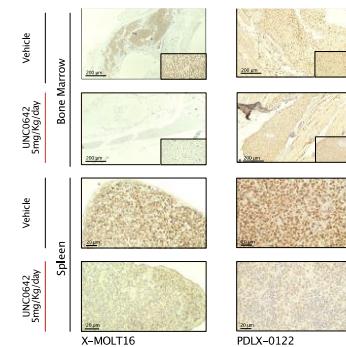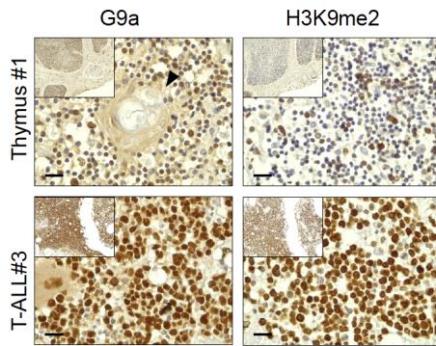
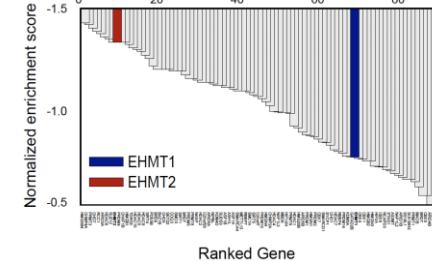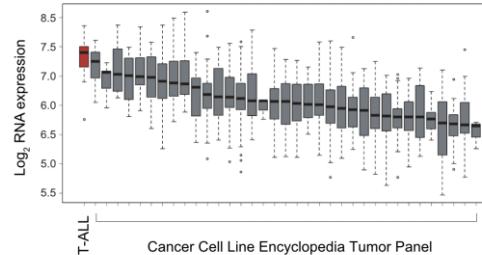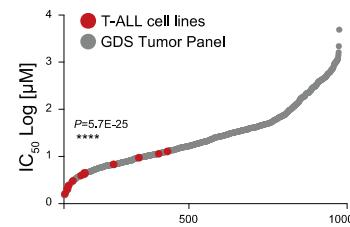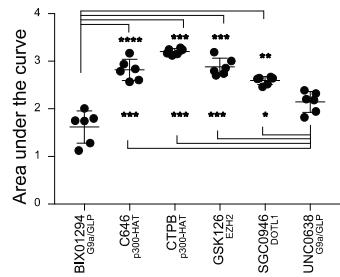*Marchesini et.al Nature Comm. 2023, in revision*
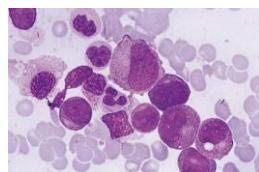
Roti G et al., Blood 2007

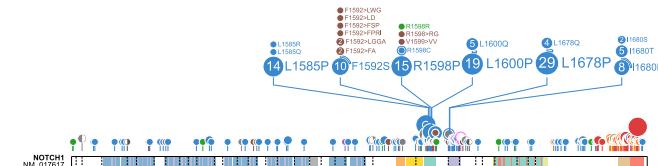# Epigenetic Targets and T-ALL

Targeting the Protein Methyltransferase G9a in T-ALL

Chemical Genomics: Elucidating Biological Systems with Small-Molecule Compounds

Individual Level to Tailor Therapy to Each Patient

Phenotype of Interest

Forward Hypothesis-generating

Gene/Protein of Interest
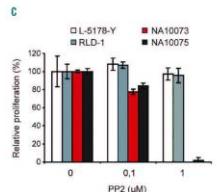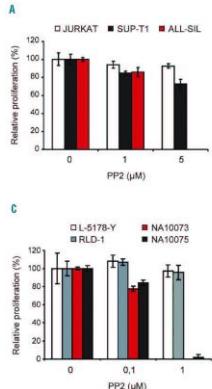
Reverse Hypothesis-generating

Small Molecule Library

Targeted Small Molecule

Drug Response Profiling Platform Development

(Adult-2014)
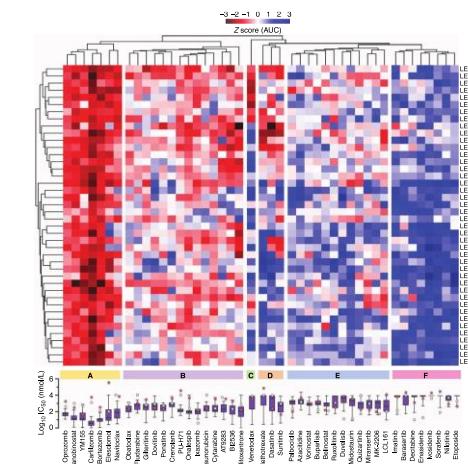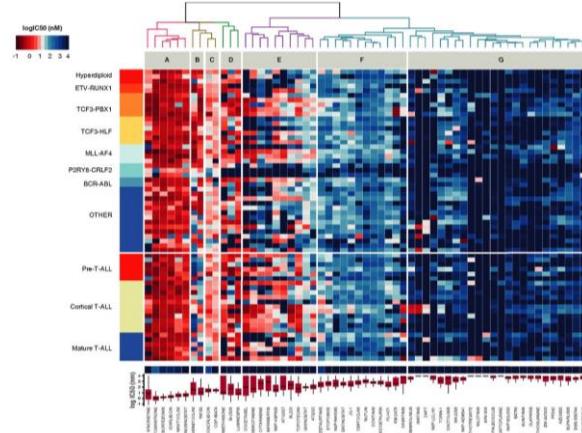
In silico

(Adult-2017)
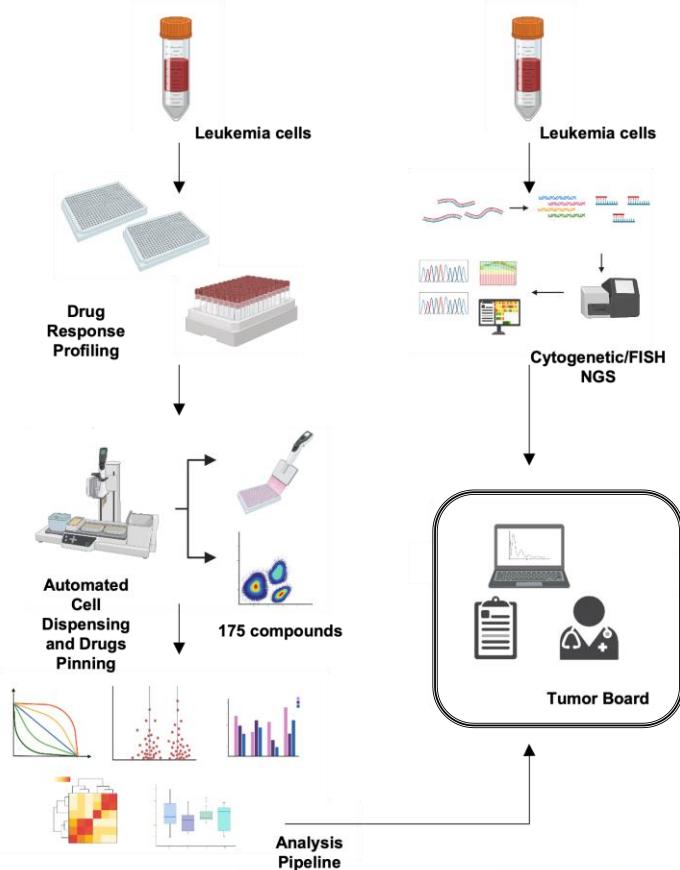
Ex-vivo

(Pediatric-2022)

Ex-vivo

Wang H. Cancer Discovery; 2022: 517-533
Blood. 2017;129(11):e26-e37
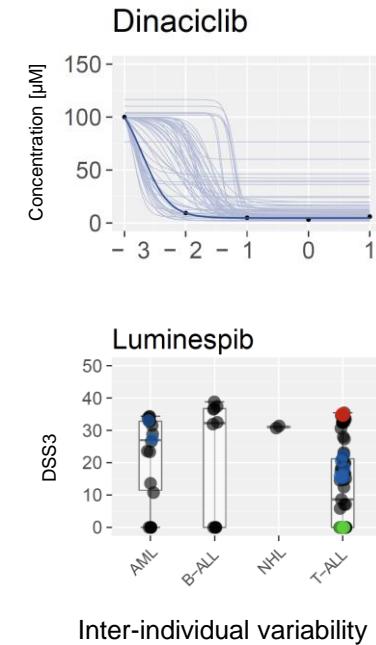*Haematologica. 2014 Jan; 99(1): 85–93.*
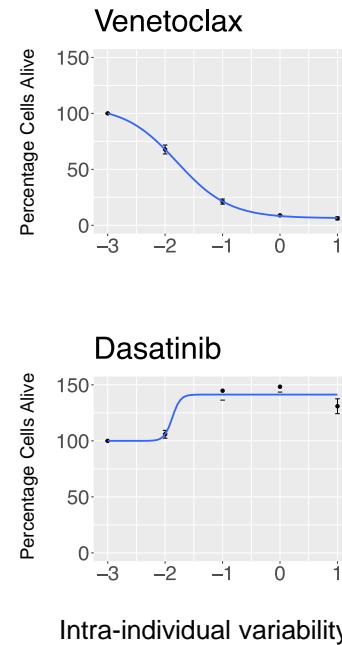
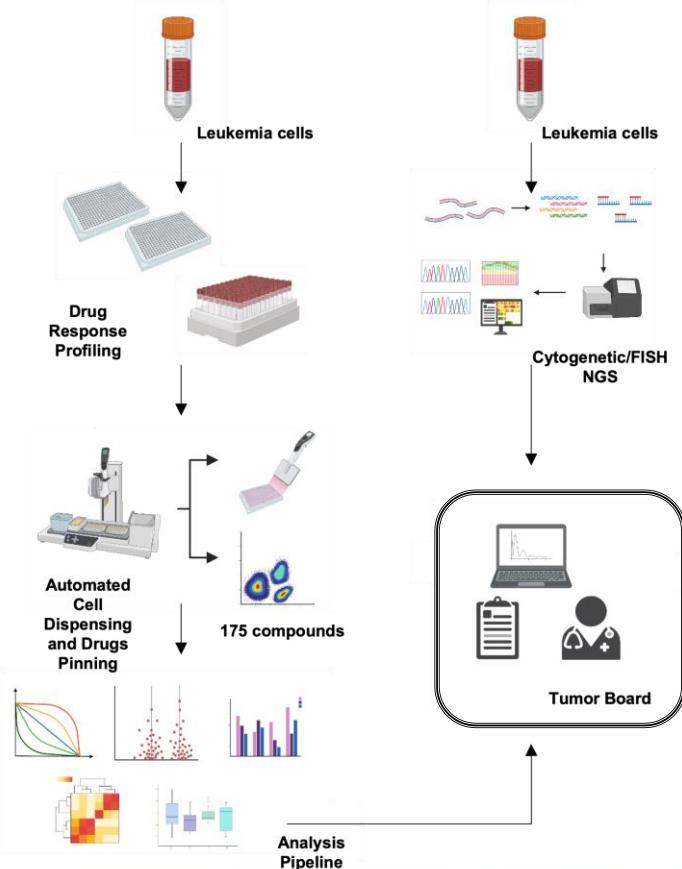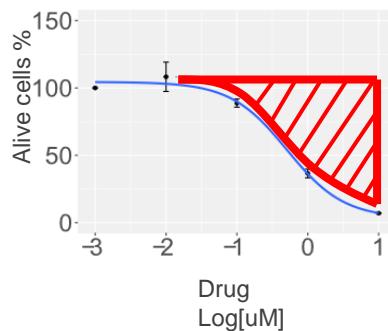# Drug Response Profiling Platform at THEC UNIPR

Dose Response

Inter-individual variability

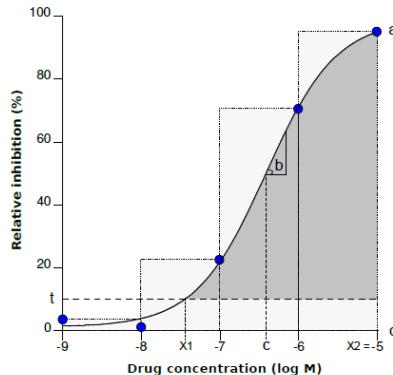*THEC Lab data*

Drug Response Profiling Platform at THEC UNIPR

THEC Lab data

Drug Sensitivity Score (DSS)

$$DSS \propto \int_{R \geq A_{min}} R(x)dx = I(IC_{50}, Slope, R_{min}, R_{max}, A_{min})$$
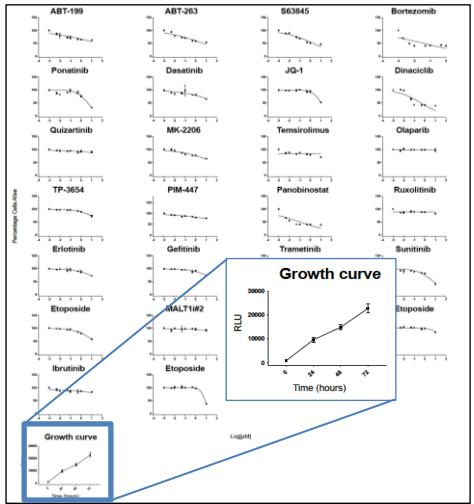

3 steps of normalization:

- <u>DSS1</u>: the area under the drug response curve is normalized with the total area.

- <u>DSS2</u>: DSS1 is normalized with log10 of max response to normalize the effect of the maximal compound response, which may be due to off-target effects.

- <u>DSS3</u>: DSS2 is normalized with concentration range, to emphasize the drugs that show a response at low concentrations.

→ Pre-defined minimum activity level (set to 10%)
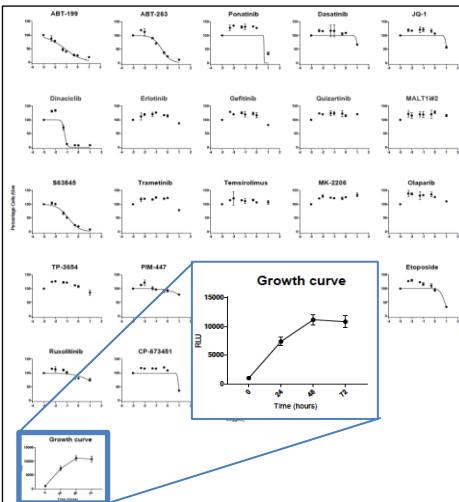→ Rmax, Slope, IC50, Rmin, and Amin

➢ **Selective DSS (sDSS): DSS (sample) – DSS (healthy controls)**

➢ **Differential DSS (dDSS): DSS (sample) – DSS median (same disease)**

DSS → 0	Resistance
DSS → ∞	Sensitivity

THEC Lab data

DRP Calibration: [*Ex Vivo* Colture] and [Drug's Response]
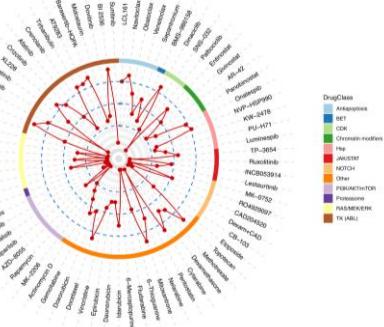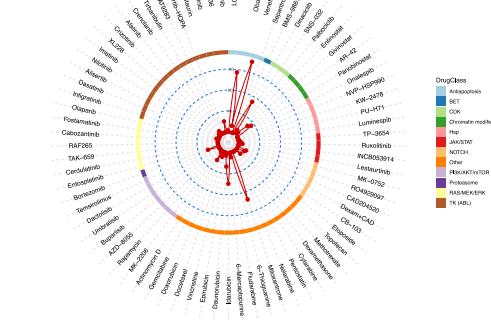

Patient's BM or PB Serum
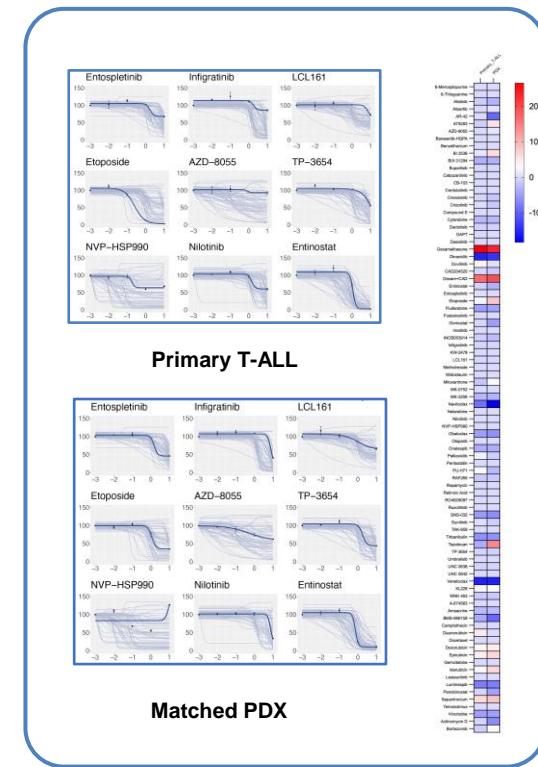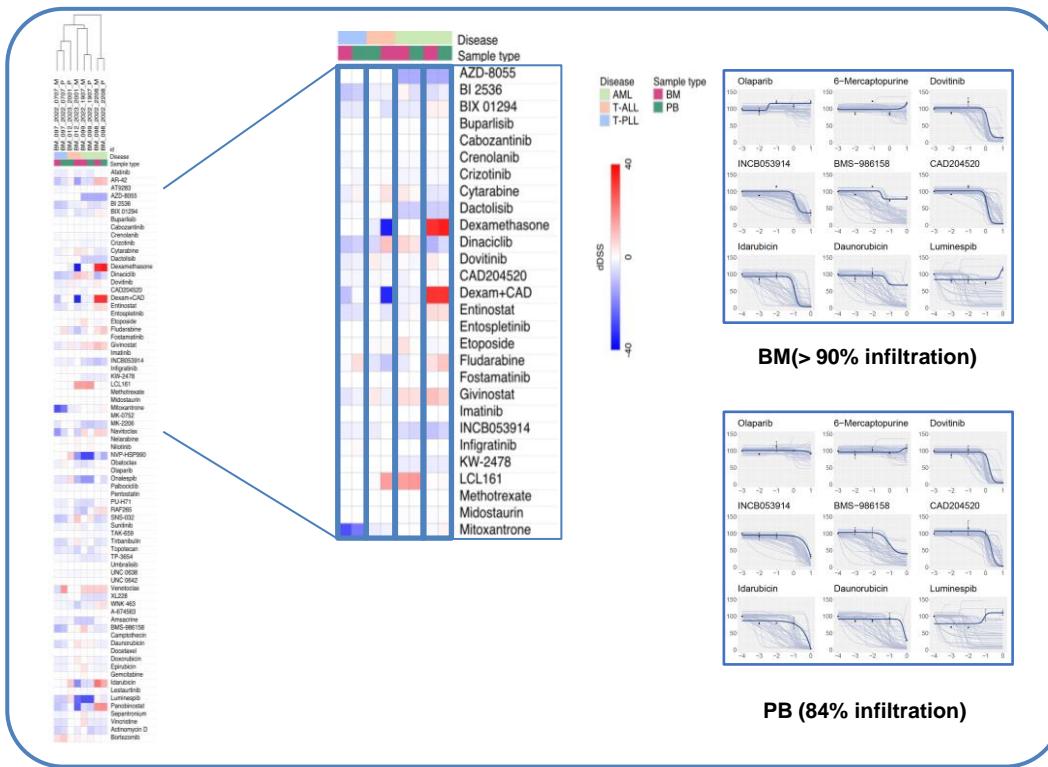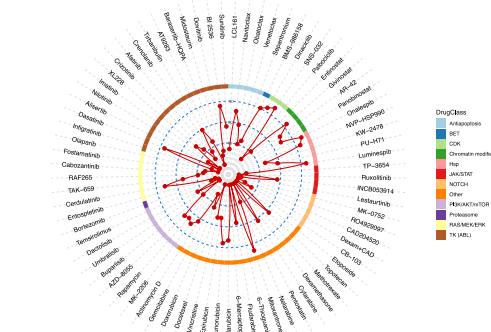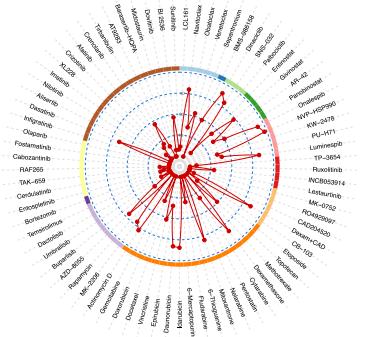
Correlation AUC vs DSS
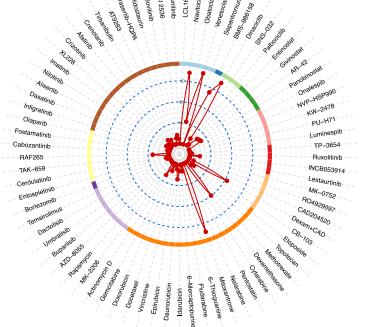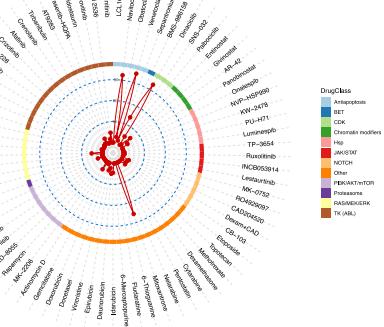
DRP Calibration: [BM vs. PB] and [Primary vs. PDX]

BM(> 90% infiltration)

PB (84% infiltration)

Primary T-ALL

Matched PDX

THEC Lab data

Repetitive Pattern of DRP Response

Distinct Patient Clusters Defined by Differential *ex vivo* Drug Sensitivity

41 T-ALL | 24 ETP | 17 nETP
30 Male | 11 Female

11 HOXA | 5 TAL/LMO| 2 TLX1 | 3 TLX3
19 Unclassified | 1 N/A

33 Relapsed | 5 Refractory
3 Diagnosis

THEC Lab data
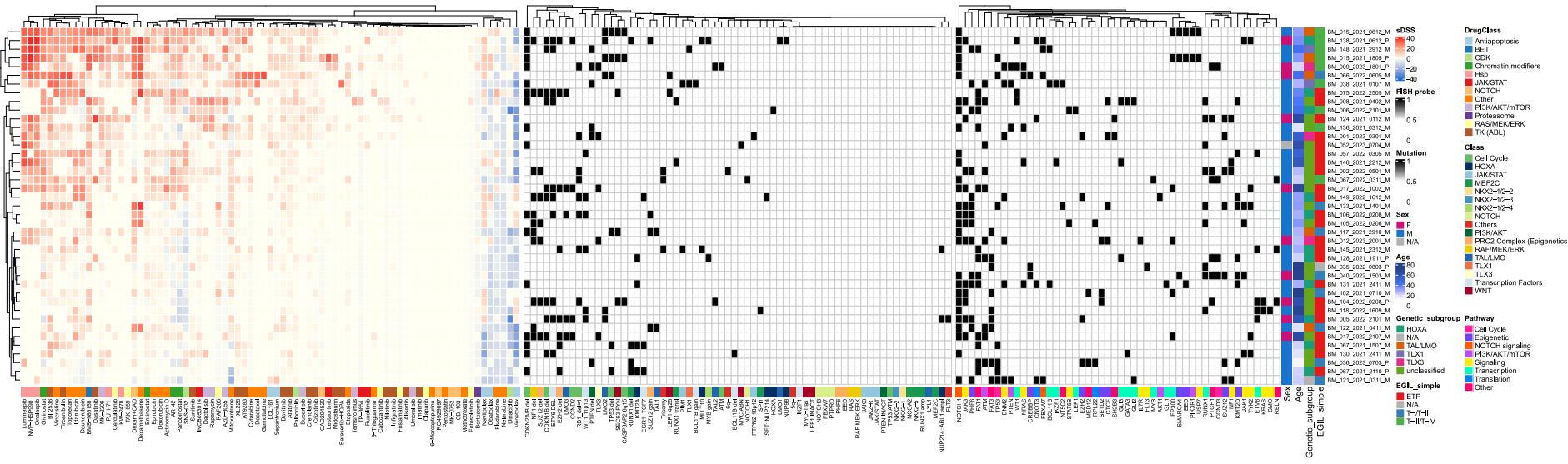
Distinct Patient Clusters Defined by Differential
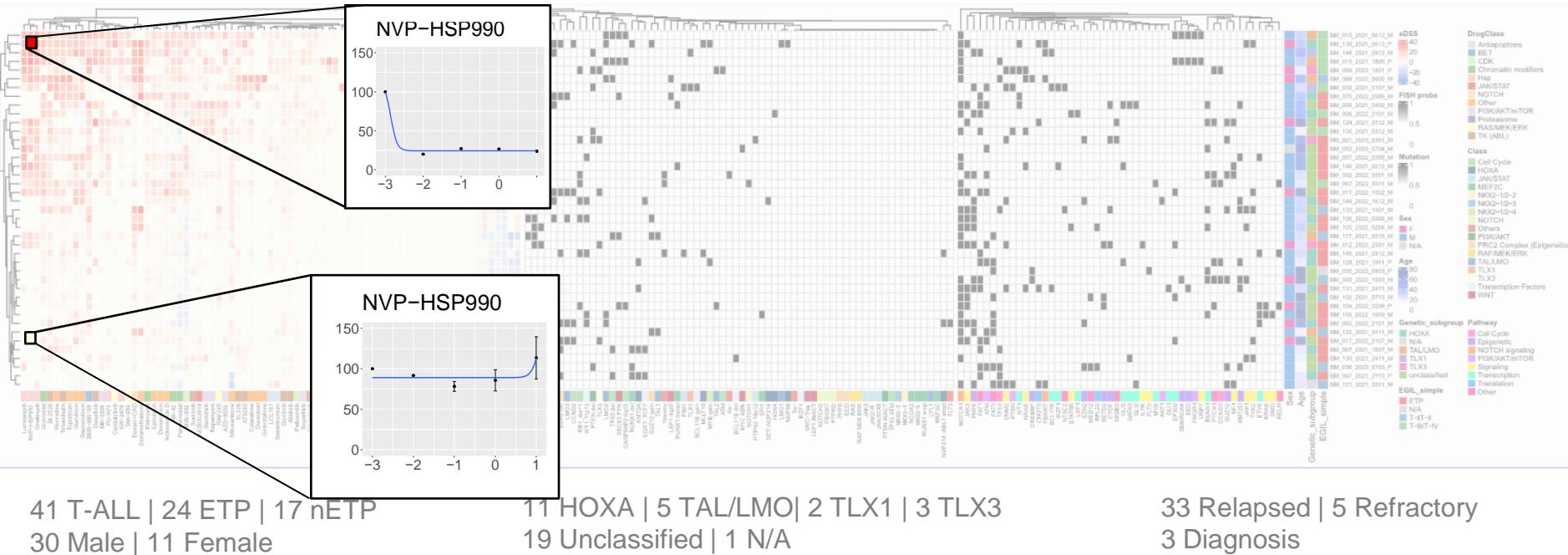# *ex vivo* Drug Sensitivity

41 T-ALL | 24 ETP | 17 nETP
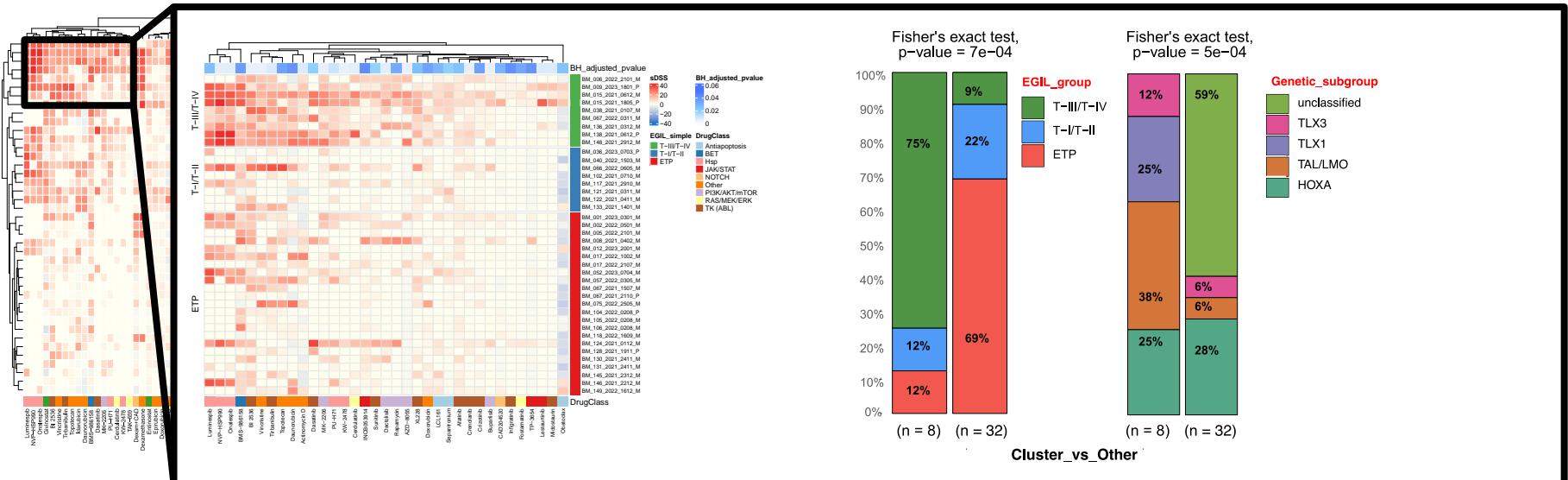30 Male | 11 Female

11 HOXA | 5 TAL/LMO| 2 TLX1 | 3 TLX3
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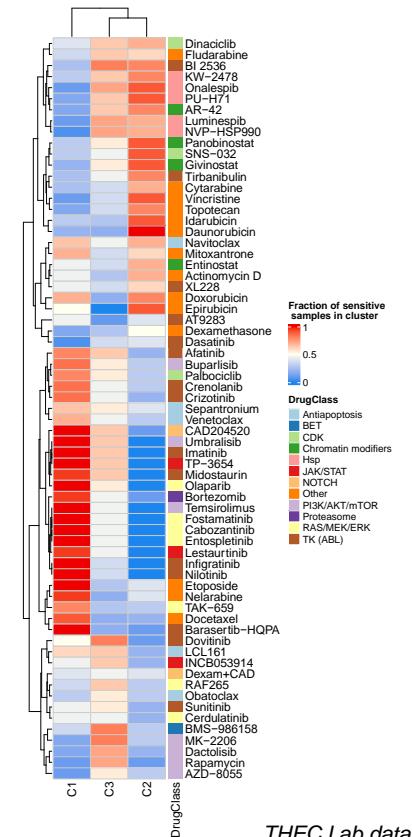33 Relapsed | 5 Refractory
3 Diagnosis

THEC Lab data

Distinct Patient Clusters Defined by Differential *ex vivo* Drug Sensitivity
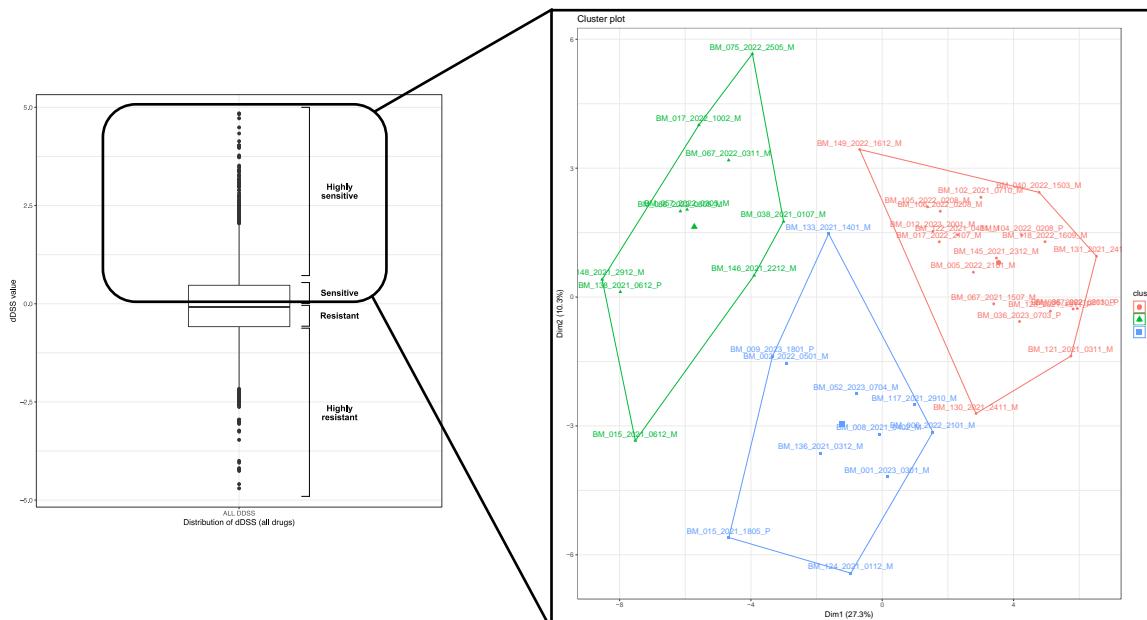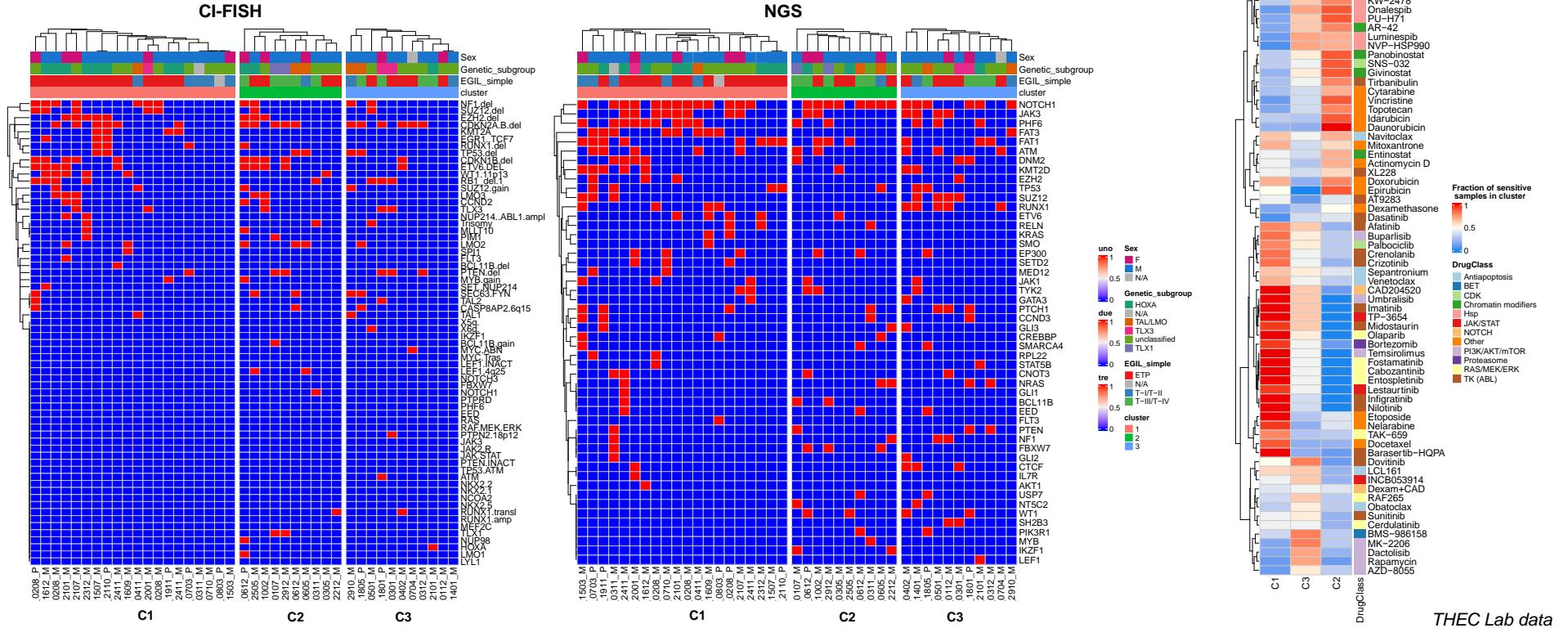

THEC Lab data

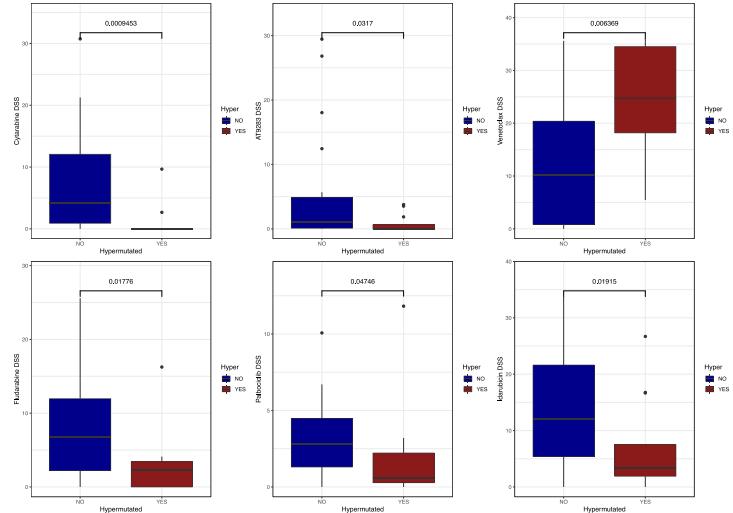# Distinct Patient Clusters Defined by Differential *ex vivo* Drug Sensitivity

THEC Lab data

Distinct Patient Clusters Defined by Differential *ex vivo* Drug Sensitivity

THEC Lab data

Recurrent Mutations Identify Pattern of Drug's Response
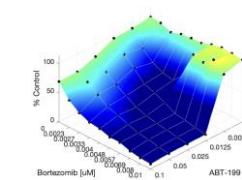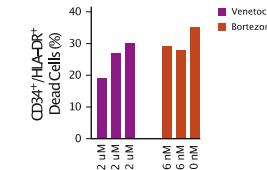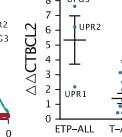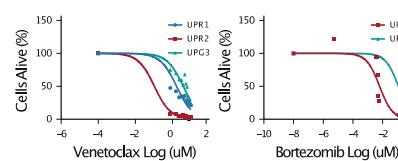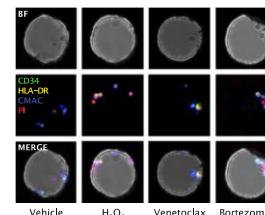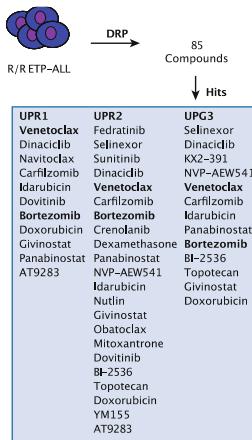
Hyper-mutation >7

SNV association

THEC Lab data
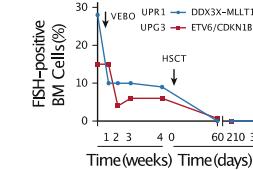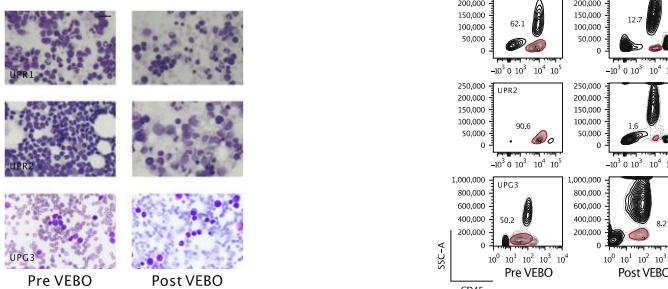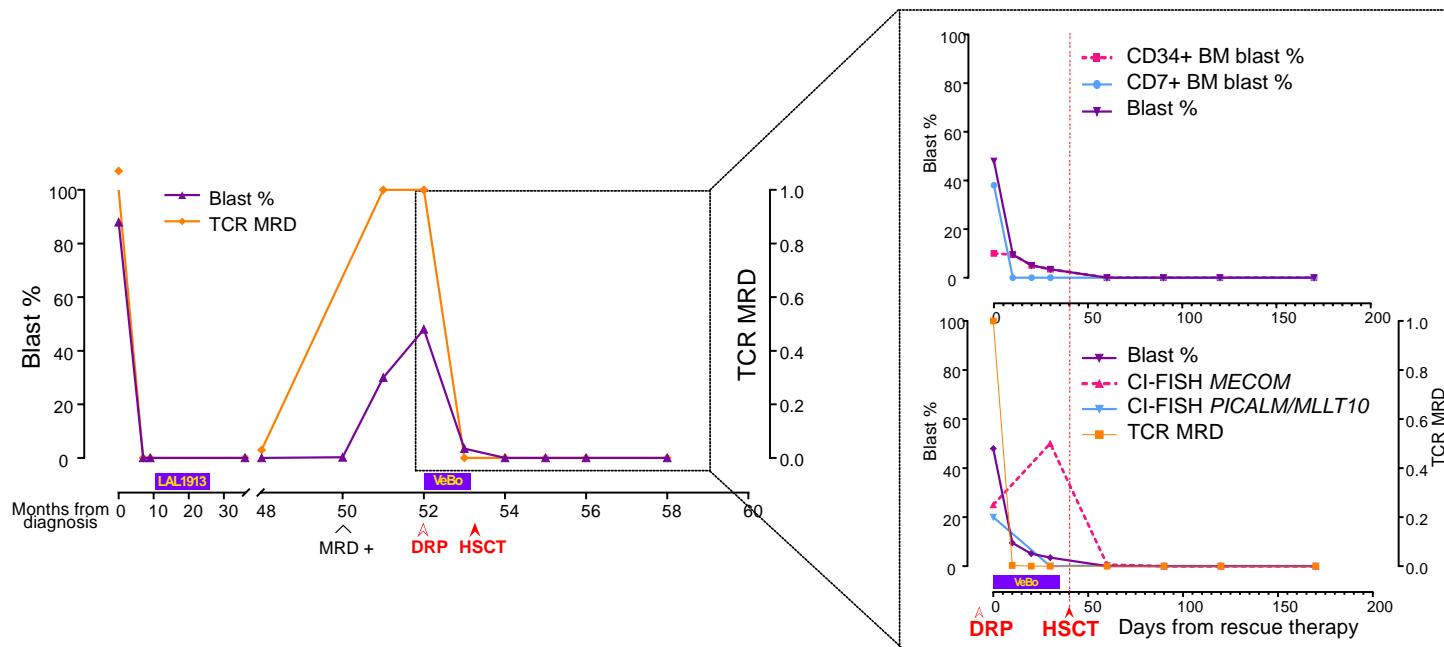
Venetoclax and Bortezomib in T-ALL
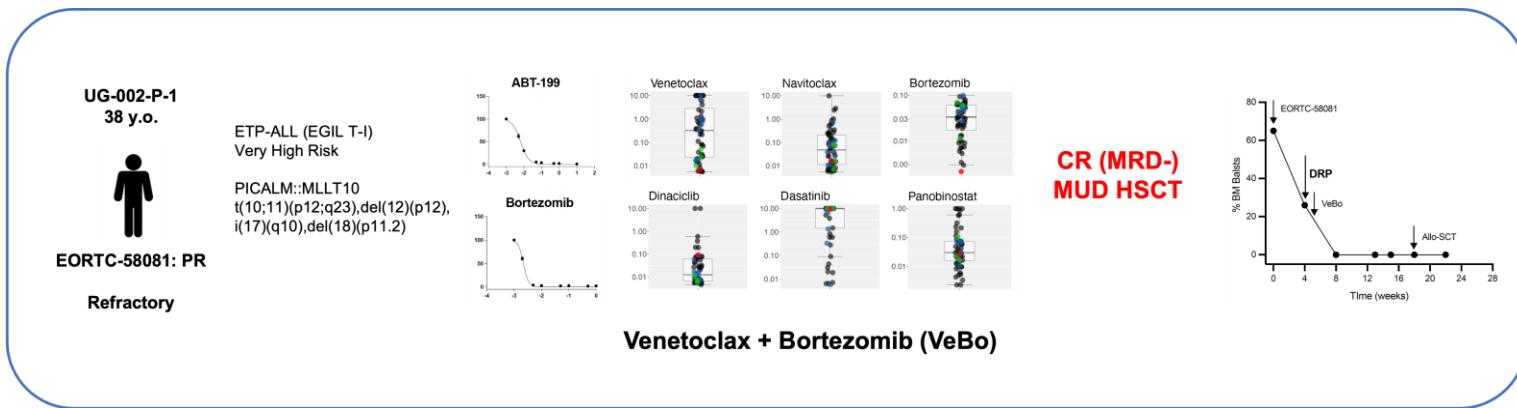
VEBO: a Continued Clinical Experience in R/R T-ALL

VEBO: a Continued Clinical Experience in R/R T-ALL

THEC Lab data

Developing N-of-1 Strategies for R/R T-ALL

THEC Lab data

Developing N-of-1 Strategies for R/R T-ALL

THEC Lab data
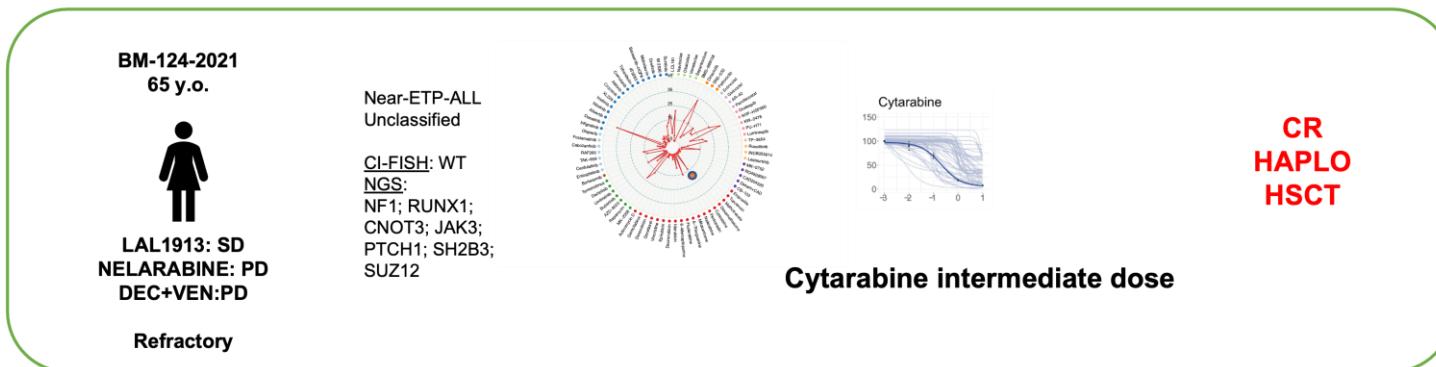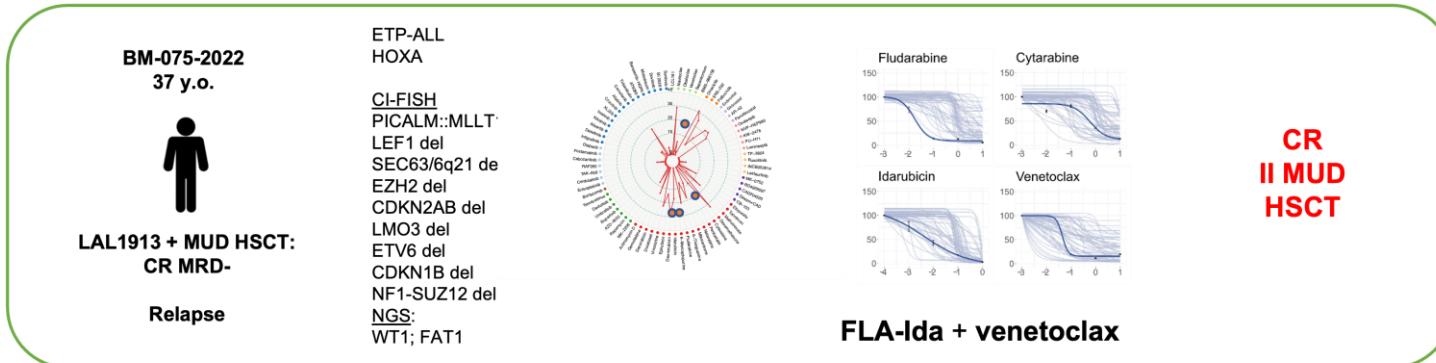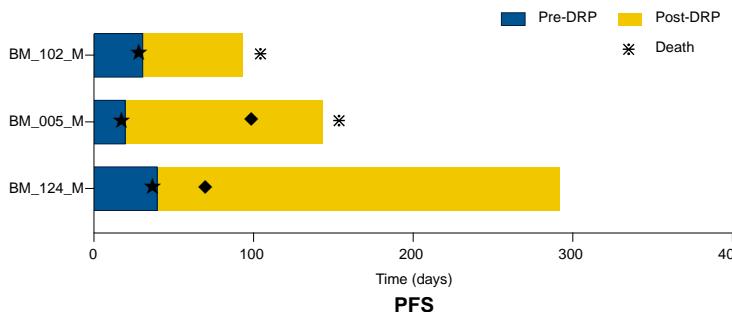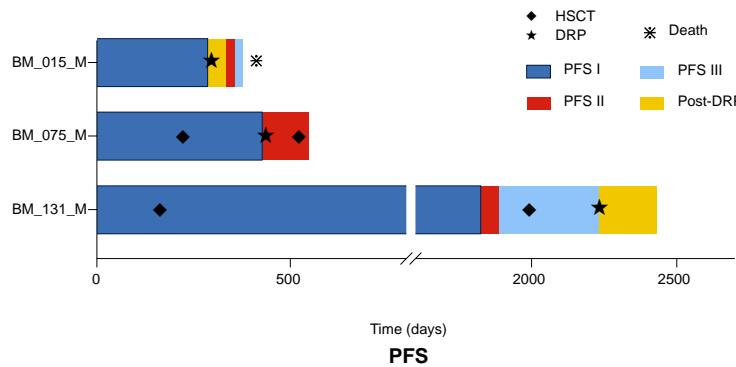
DRP Potential Clinical Applications

Alternative to Nelarabine Bridge to Transplant

Palliative care

2nd Transplant

2nd , 3rd Relapse & Intersection with CAR-T

THEC Lab data

To Do List for Functional Precision Medicine

- Limits for pre-analytical variability

  o Shipping (*max time before DRP 48h*)

  o Handling (*efficient BM aspirate*)

  o Tissue type (*PB/BM and serum*)

- Report on clinical correlation with FMP assay

  o Case report

  o Clinical trial

- FMP by Pharmaceutical company

  o e.g. CB103

- Share the data (*Patient Characteristics - CRF*)

fondazione GIMEMA onlus
per la promozione e lo sviluppo della ricerca scientifica
sulle malattie ematologiche. **FRANCO MANDELLI**

Advancing chemical and genomic strategies for relapsed/refractory T-ALL and ETP-ALL

GIMEMA ALL 2720

CT.gov identifier: NCT04582487

Conclusions

» Functional *ex vivo* drug profiling and chemogenomic screens can facilitate:

– Identification of actionable targets at single patient level

– Identification of molecular driver at population level

» Reverse this information to identify new disease biomarkers

» Probe individual pathway dependencies

– Intratumor heterogeneity and its impact on drug response

– The microenvironment as determinant of drug response

» Discovery of clinically relevant drug-repositioning opportunities

» N-of-1 clinical trial

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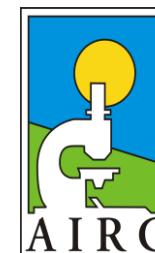Valentina Bardelli, PhD

UGhent

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Prof. Steven Goossens, PhD

Tim Pieters, PhD

All the collaborating Hematology Centers

8° WORKSHOP IN EMATOLOGIA TRASLAZIONALE

DELLA SOCIETÀ ITALIANA DI EMATOLOGIA SPERIMENTALE

Firenze - Auditorium CTO - A.O.U. Careggi, 22-23 giugno 2023

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