# *Animal models*

2012...2015.

T-Cell Lymphomas: We are illuminating the darkest of tunnels

April 27-29, 2015
Bologna

GIORGIO INGHIRAMI
Weill Cornell Medical College, New York, NY
Center of Experimental Medicine and Research (CeRMS) University of Turin

# *Why we need reliable pre-clinical models?*

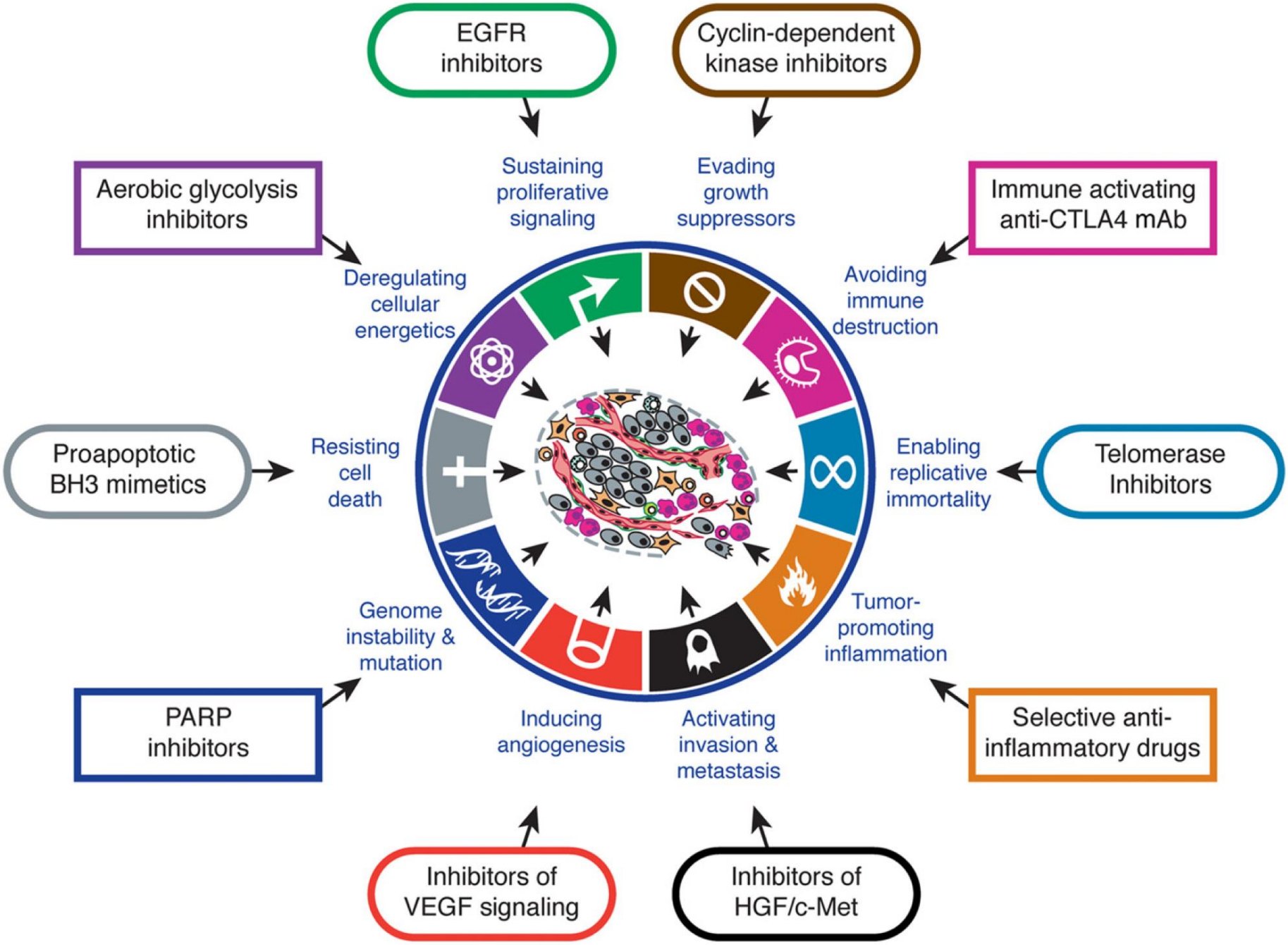
- Only **a handful number of compounds** reaches the clinical arena (1 of 10,000).
- **Small numbers of poorly characterized tumor cell lines** that inadequately recapitulate human disease
- 'Xenografts do not predict for human effects', with **90% of novel antineoplastic drugs failing** despite antitumor efficacy in classical preclinical models.
- Conventional **GEM mouse models** express/loose target genes in all cells under ectopic/forced promoters/enhancer, often with a constitutive modality
- Xenograft and/or **GEM, PDX models do not fully recapitulate the human tumour environment**
- **Erroneous use and misinterpretation of preclinical data** from cell lines and animal models.
- A poor appreciation of pharmacokinetics and pharmacodynamics, and the use of problematic endpoints and testing strategies.
- **Preclinical testing rarely includes predictive biomarkers** that, when advanced to clinical trials, will help to distinguish those patients who are likely to benefit from a drug.

**C. Glenn Begley & Lee M. Ellis:** *Nature* **483**, 531–533 (29 March 2012)

# *What about reproducible PTCL models?*

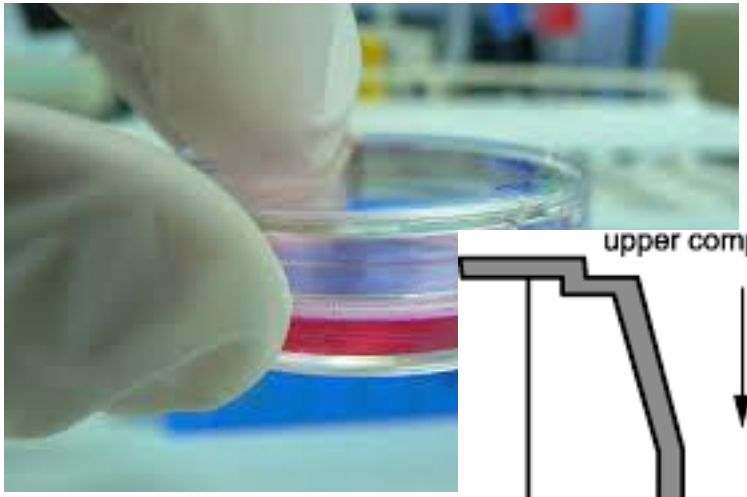
- Although several cell lines derived from T-ALL exist. Rare in vitro models for neoplastic post-thymic lymphocytes are available, with HTLV-I+, CTCL and ALK+ ALCL lines representing the exception.

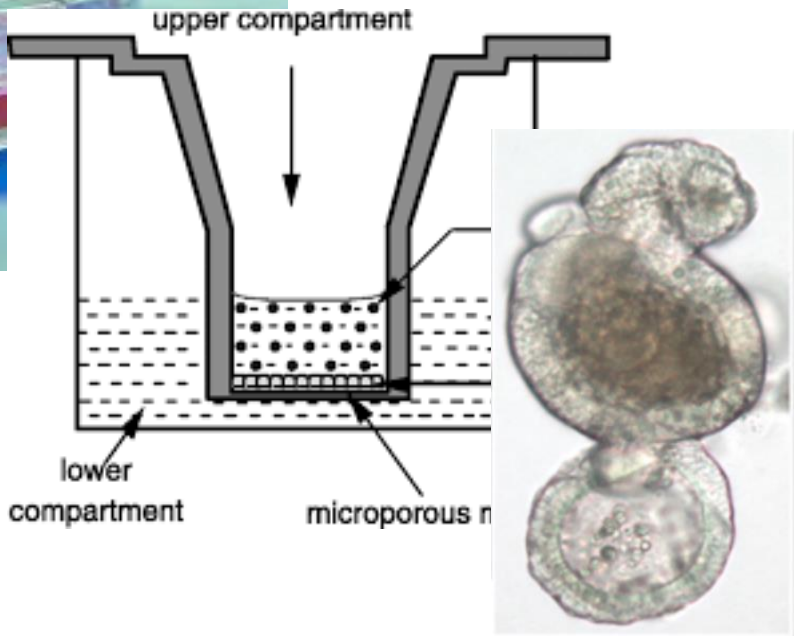
- Few spontaneous (Roquinsan) or engineered (i.e. ITK-SYK, NPM-ALK) mouse models, faithfully reproducing their corresponding human counterparts, have been successfully used to define the pathogenetic mechanisms leading to T-cell transformation and/or design and validate therapeutic protocols.



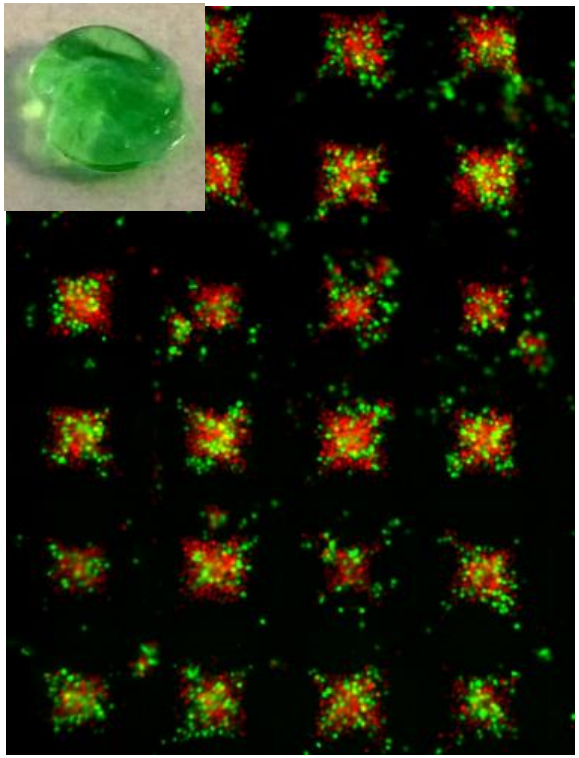

Douglas Hanahan and Robert A. Weinberg Cell. 2011, 44(5):646-74

# *Have we the right models ?: "2D versus 3D in vitro"*

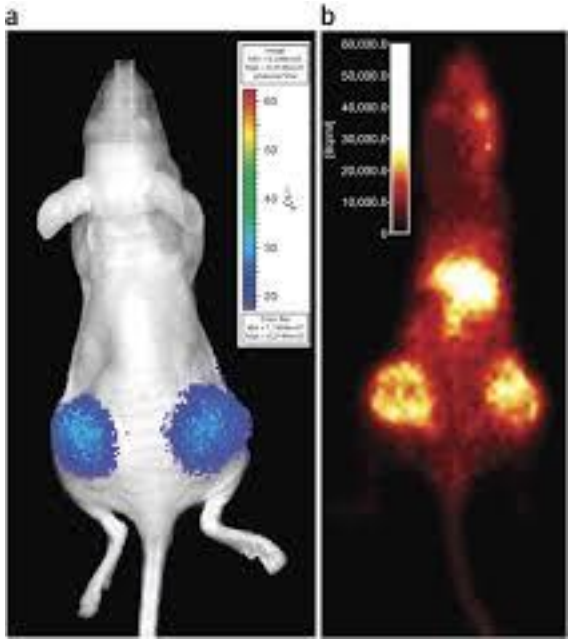


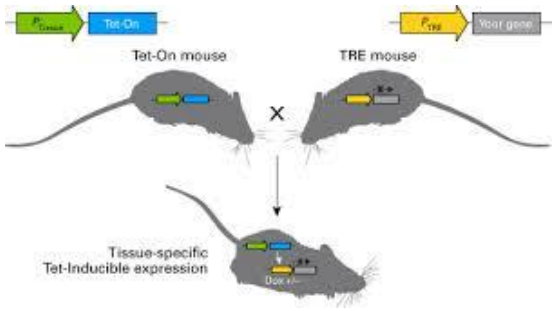


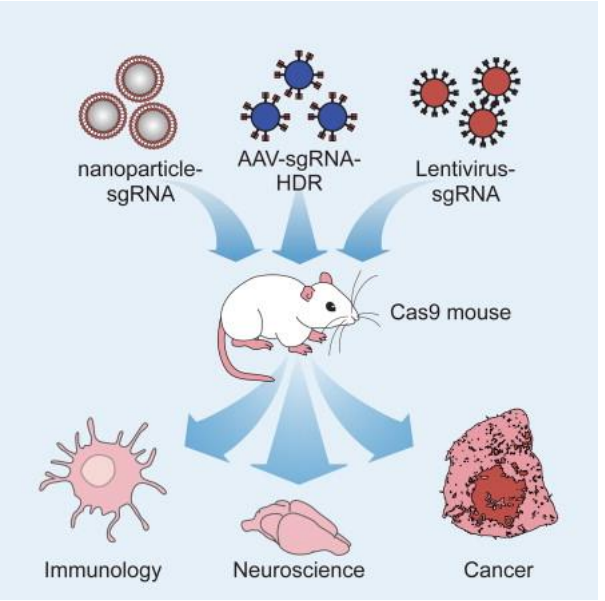


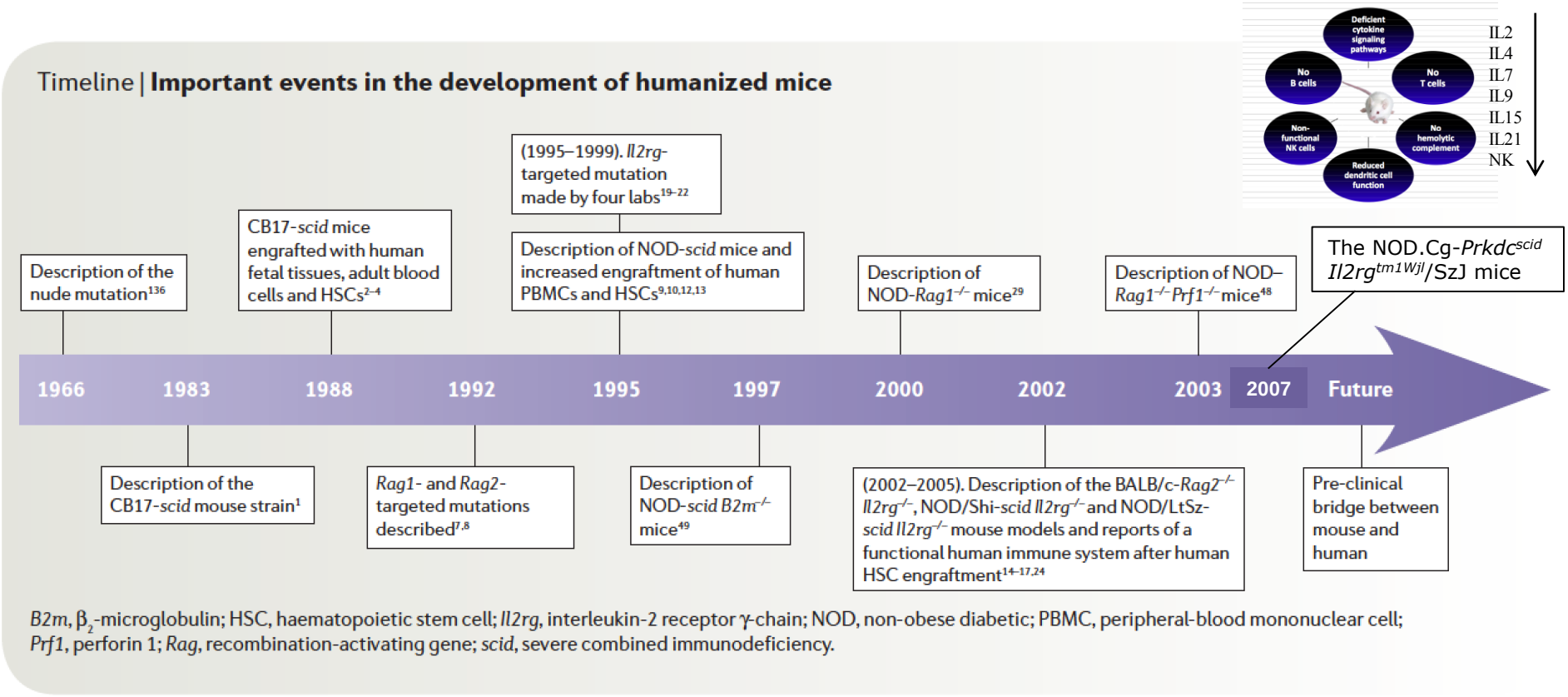
# *Rapidly evolving strategies for new mouse models*



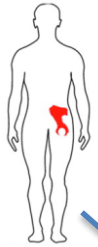



# *Humanized mouse models*

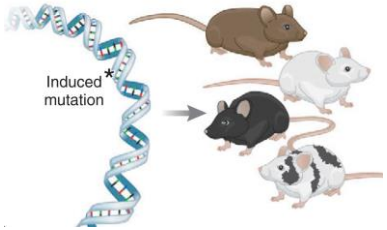



*Shultz L.D. et al Nature Rev Immunol 2007, 7, 118-130*

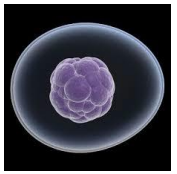
# The PDTX models



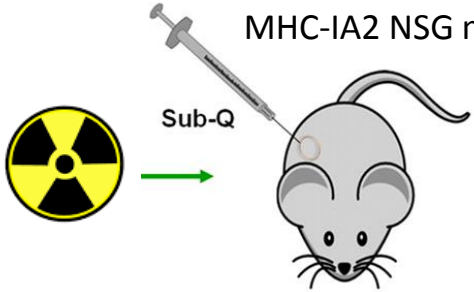

Humanized NSG mice

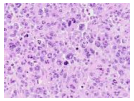



MHC-I ko
IL-6
IL-3, CSF2, Kit ligand
Class II
BAFF



MHC-IA2 NSG mice



Organoids

# NOD/scid and NSG mice have been successfully used to study human CTL

Cell PRESS

*Meyer L.H. et al 2011*

Cancer Cell
Article

**Early Relapse in ALL Is Identified by Time to Leukemia in NOD/SCID Mice and Is Characterized by a Gene Signature Involving Survival Pathways**

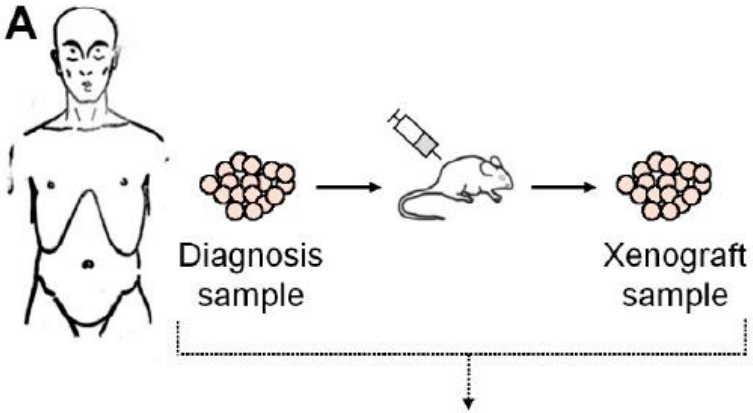
Brief Definitive Report

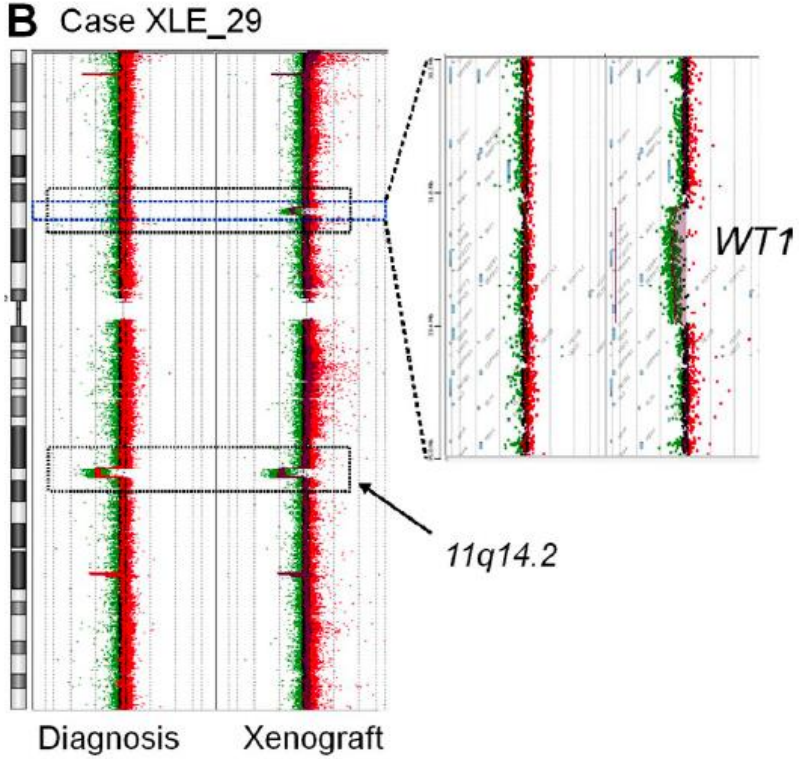
JEM *Clappier E. et al 2011*

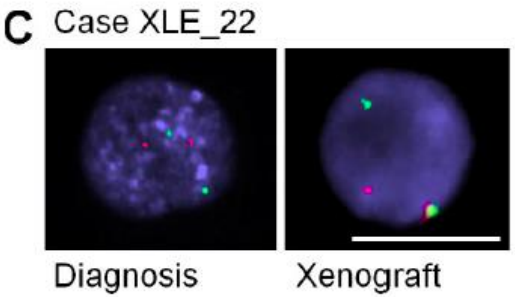
Clonal selection in xenografted human T cell acute lymphoblastic leukemia recapitulates gain of malignancy at relapse

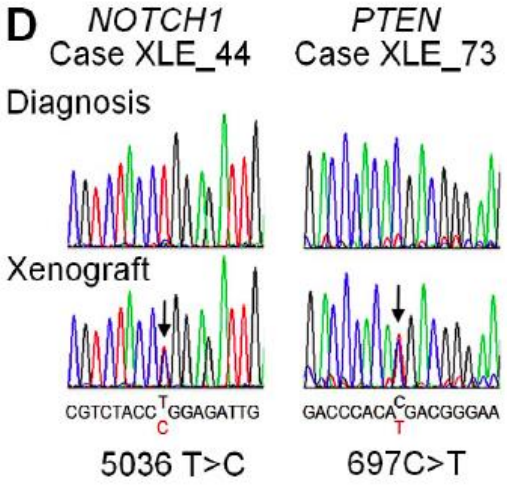
*Experimental Dermatology,* ***Krejsgaard T. et al 2010***

**A novel xenograft model of cutaneous T-cell lymphoma**



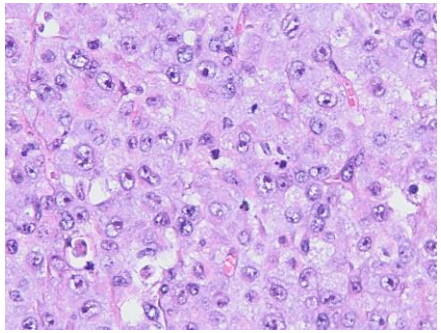



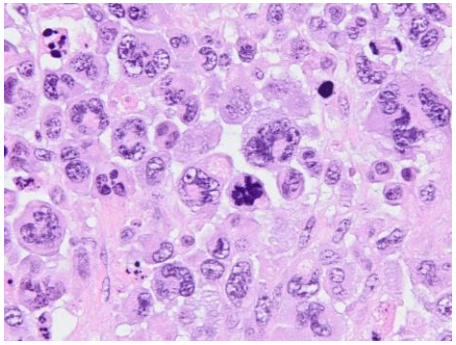




E

| Gene | Change in xenograft |
|---|---|
| *PTEN* | Deletion or mutation |
| *CDKN2A/B* | Deletion |
| *WT1* | Deletion |
| *DLEU7* | Deletion |
| *MYB* | Gain |
| *MYC* | Translocation |
| *NOTCH1* | Mutation |
| *FBXW7* | Mutation |

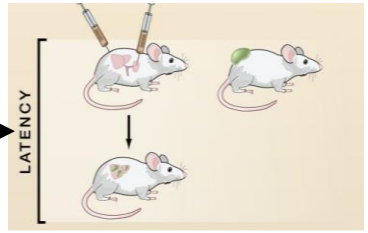
# Patient Derived Tumor Graft from fresh and/or cryopreserved tissue samples





Biobanks

Tumor fragment implants
in NSG mice




6 wks

Serial tumorgrafts ALK+ ALCL

T2

T3

T4

T5

T6

T7

8 wks

Serial Tumorgrafts ALK- ALCL

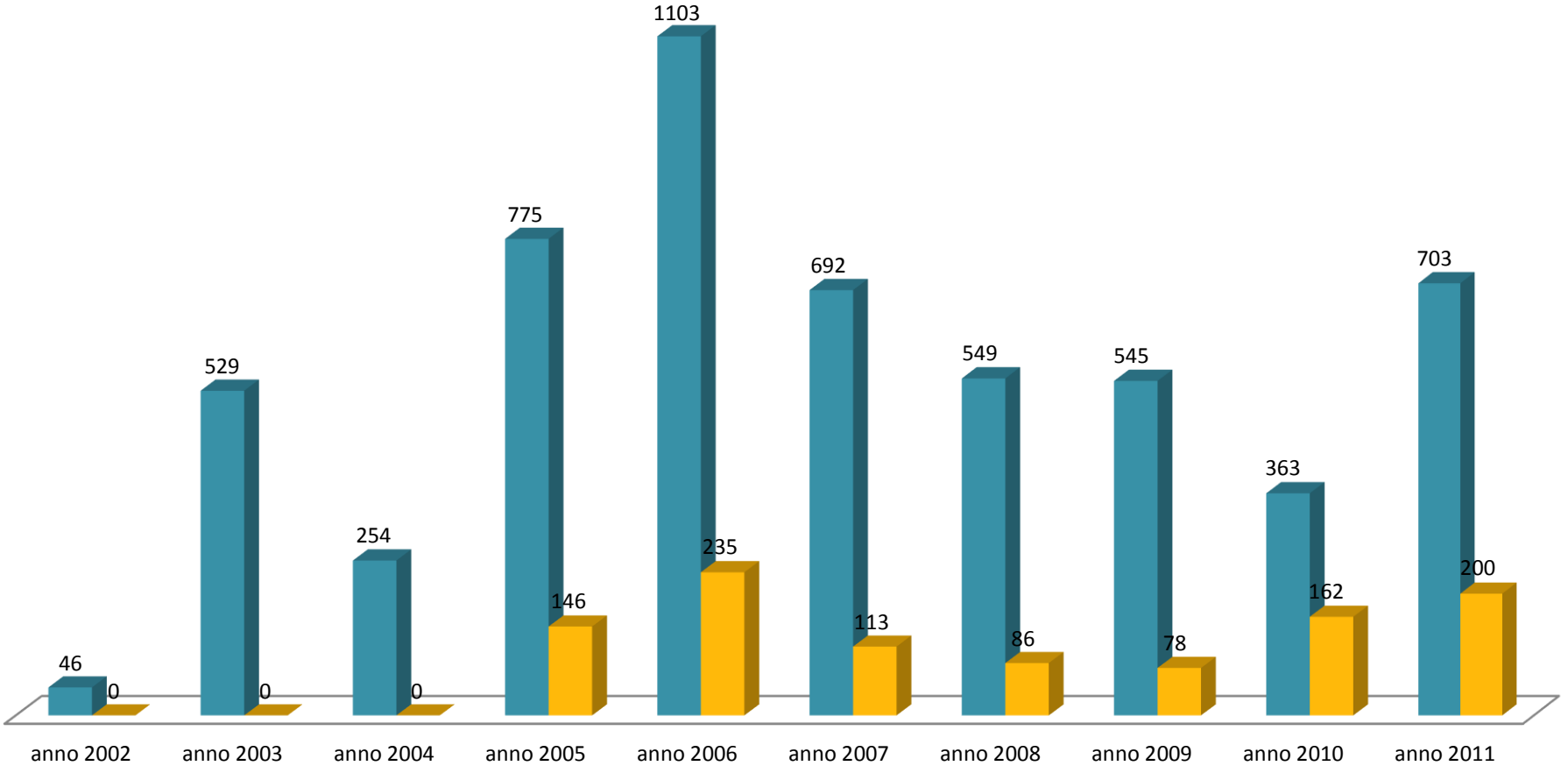
T2

T3

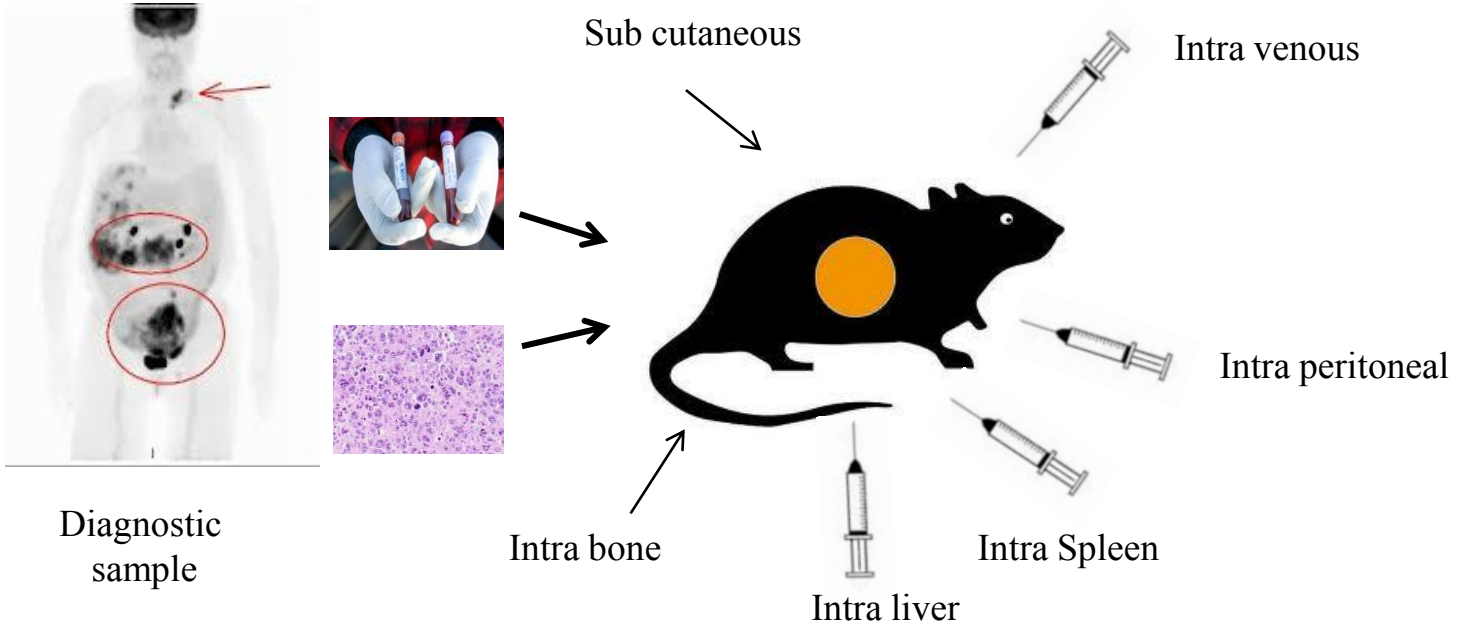
T4

T5

# *Source of cancer samples: Fresh versus viable frozen tissues?*



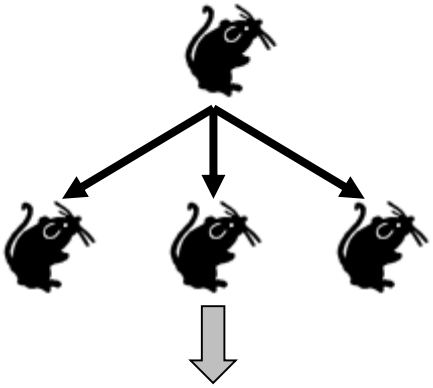
# *Can different implantation routes improve PDTX grafting?*



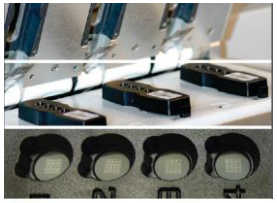
# *Precision Therapeutic Medicine: new ideas at work*

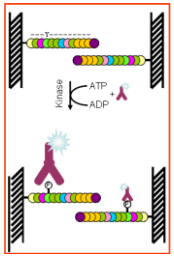

**Biorepository**

**Tumor expansion**

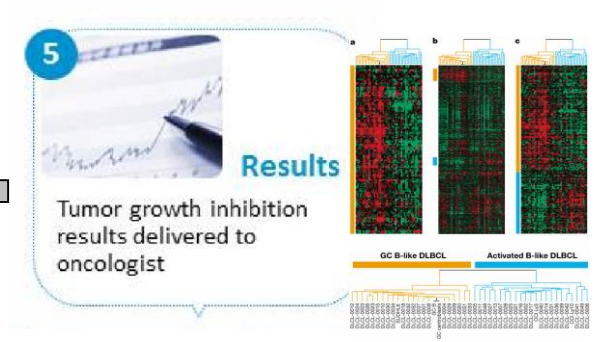



**HTP molecular stratification**





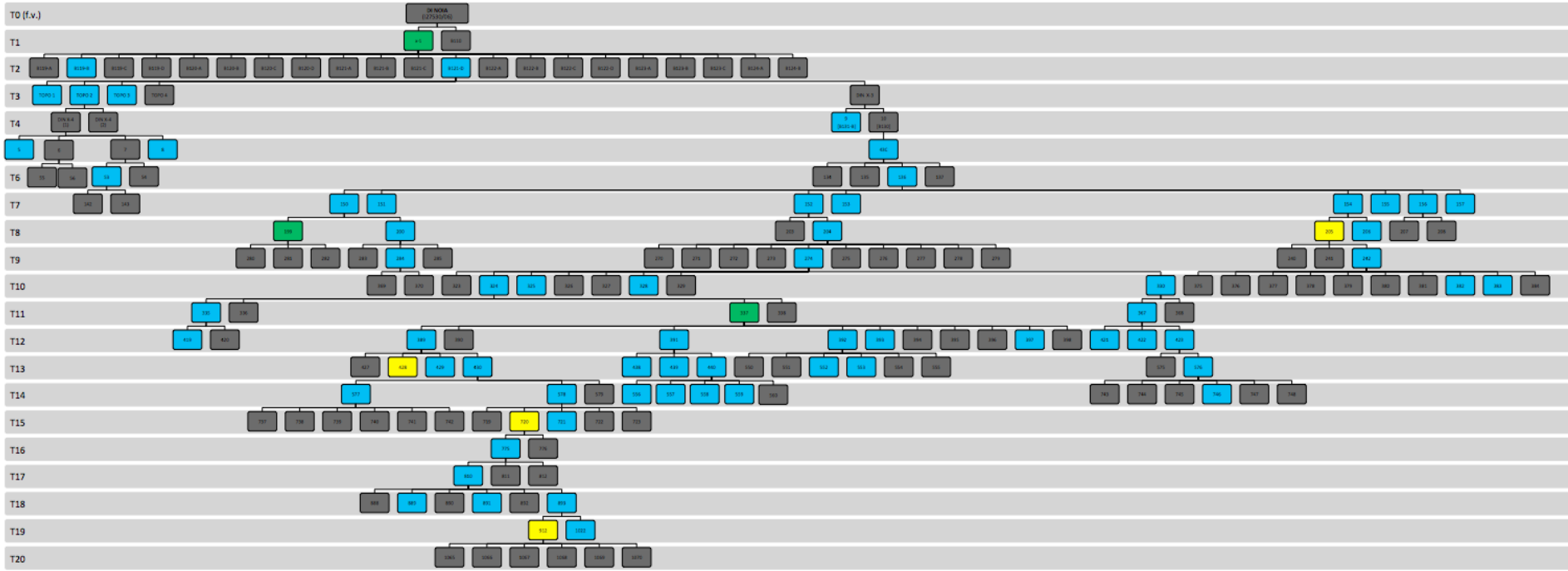

**Molecular stratifiers**

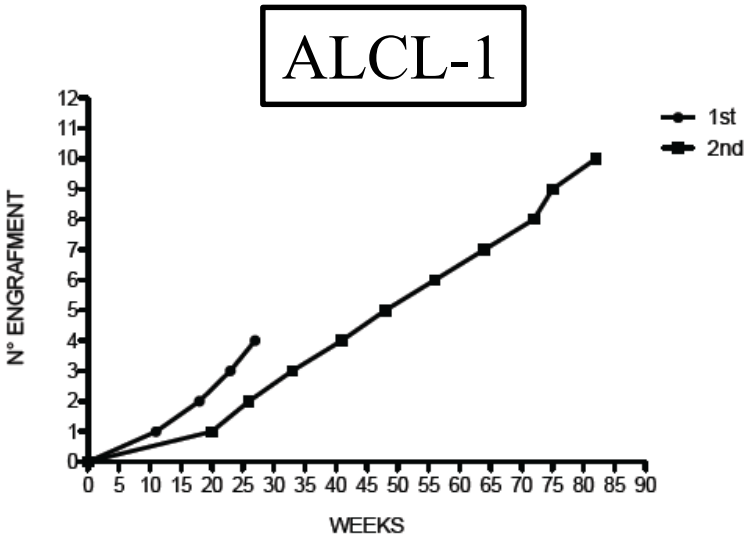

- **WES**
- **RNAseq**
- **RRBs**
- **Proteomics**

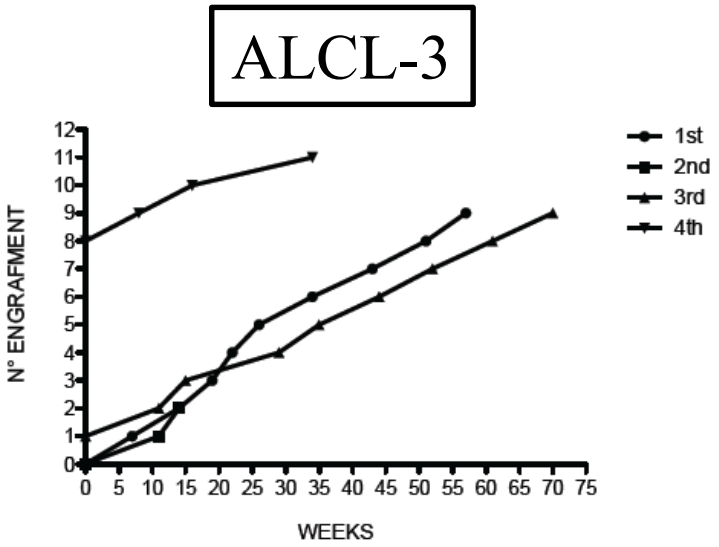
# *Representative ALCL tumorgraft expansion*

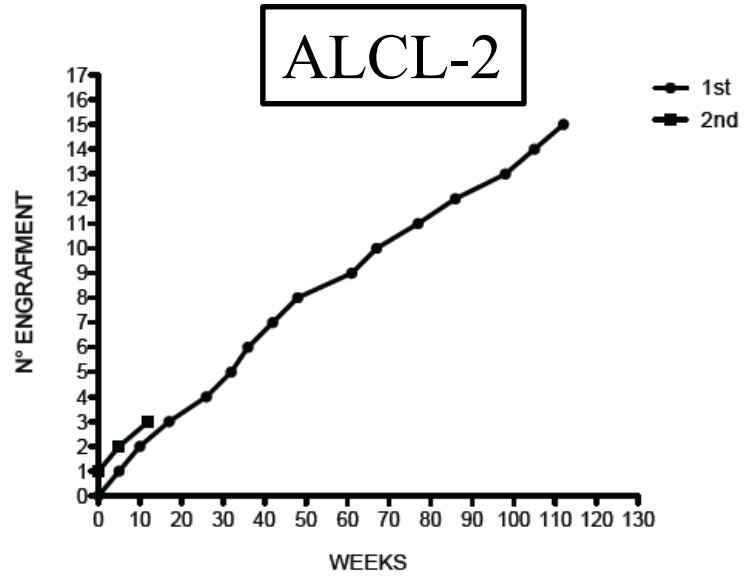
ALCL-1

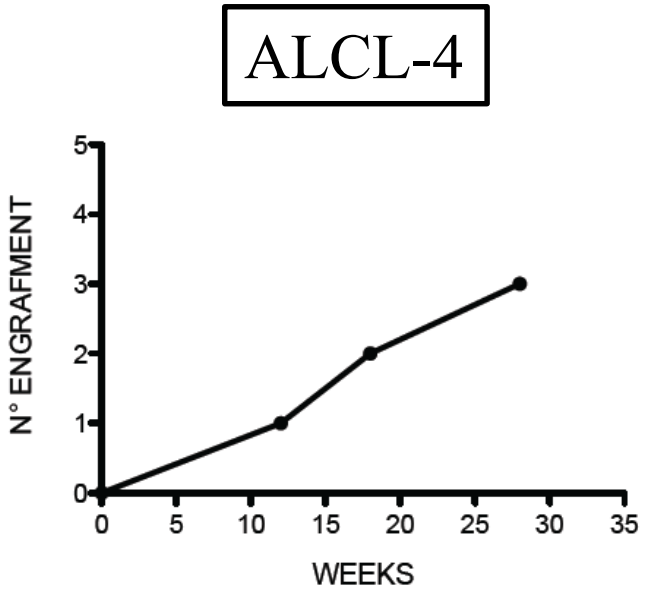


# ALCL time curves along serial tumorgraft passages

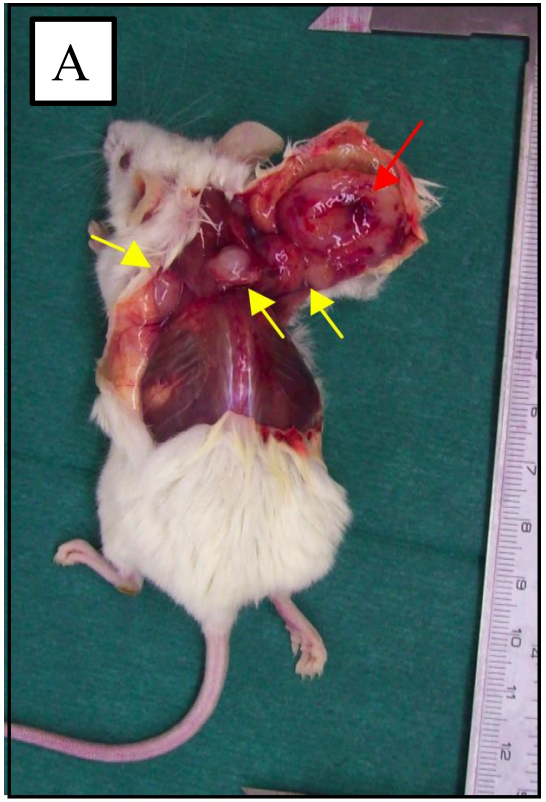


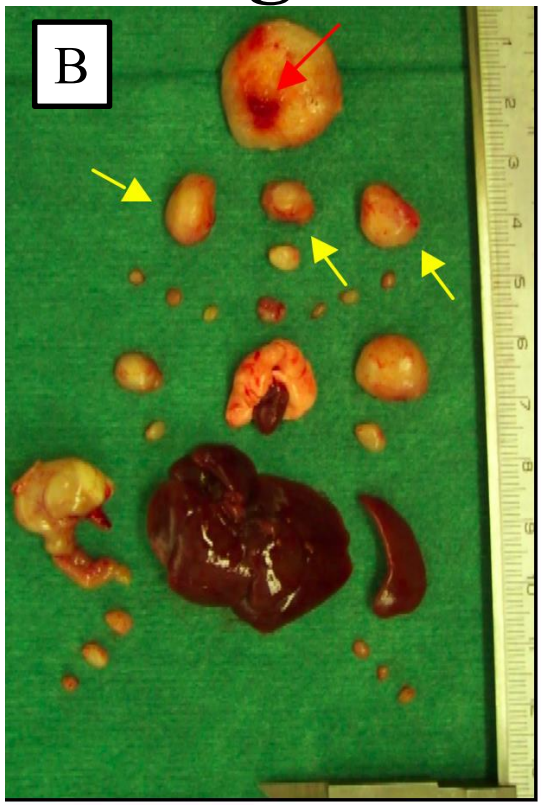



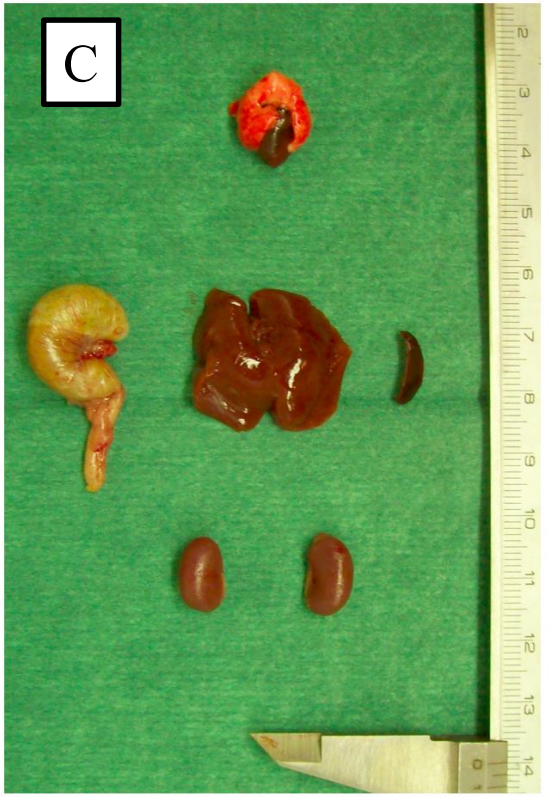



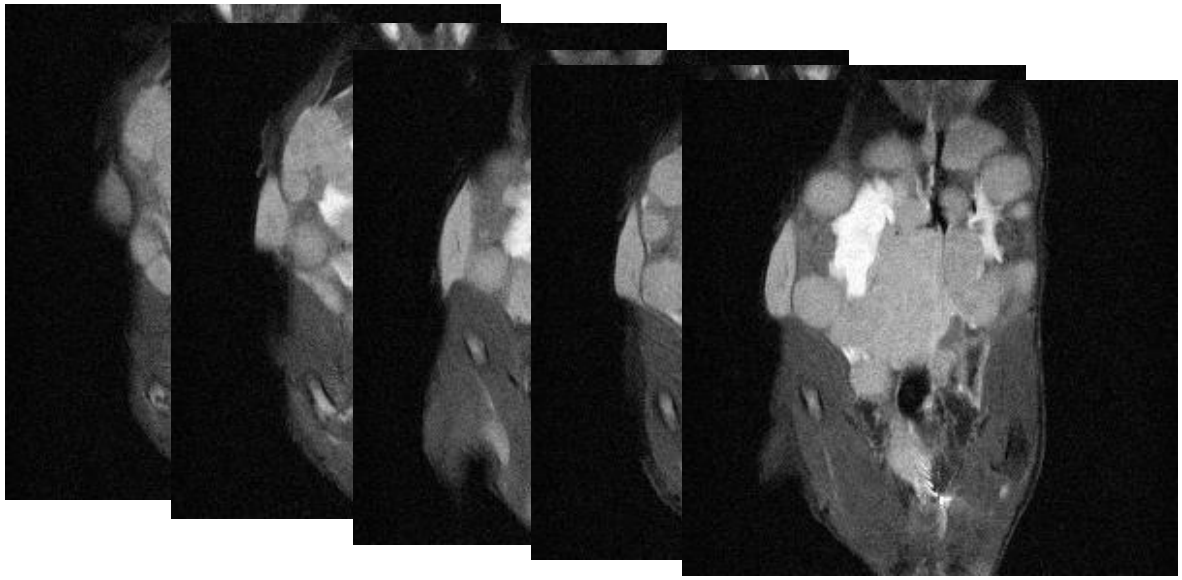
# ALCL tumorgraft on disseminate to secondary lymphoid and parenchymal organs



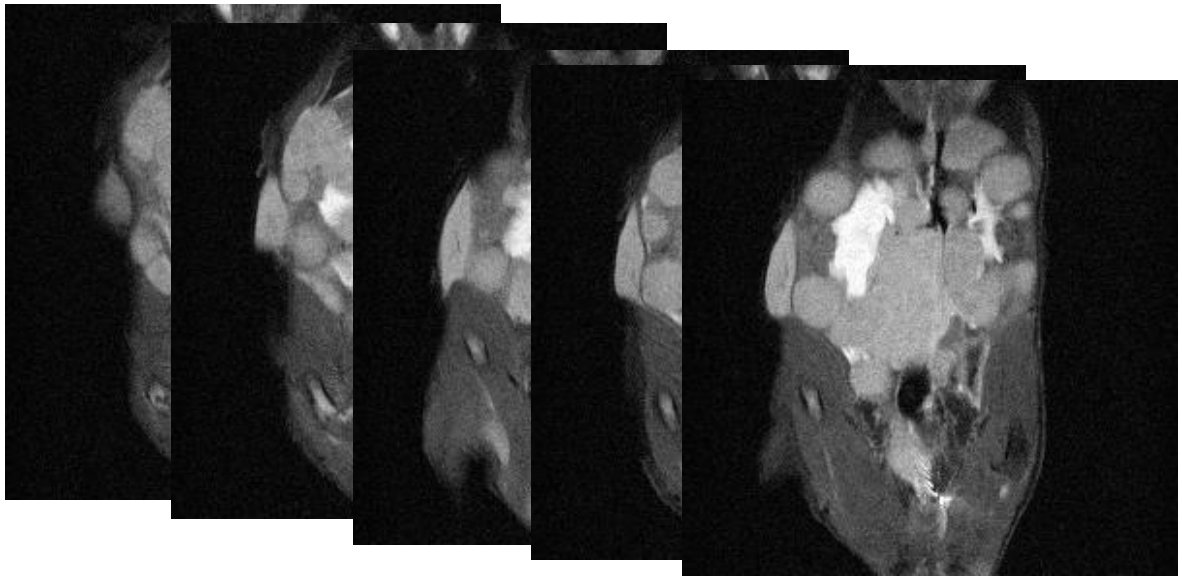



# *Total body MRI scanning and therapeutic response assessments*



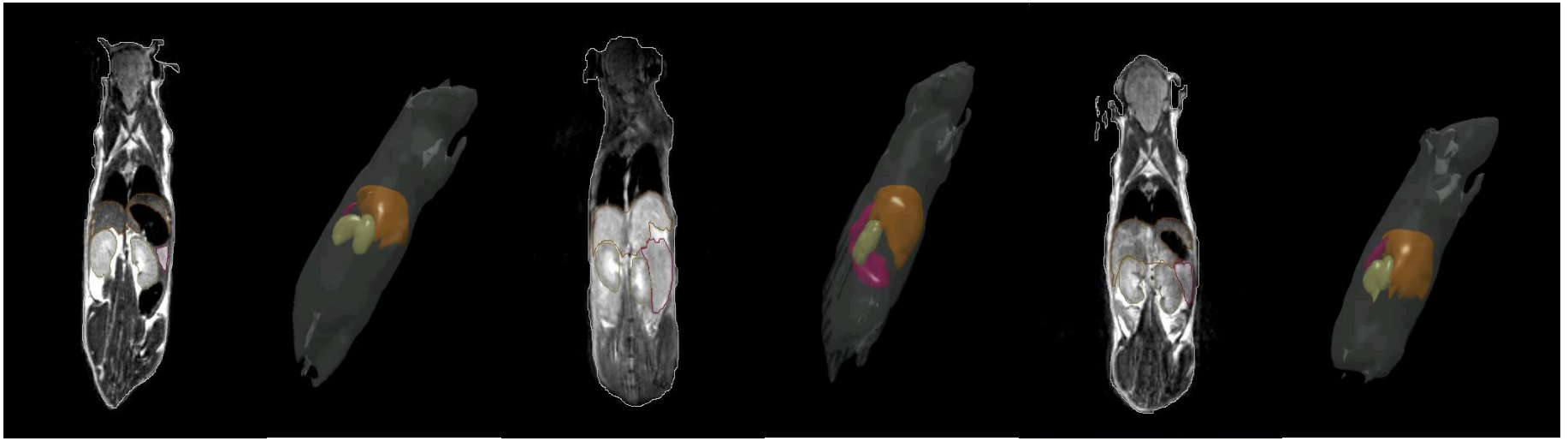
aspectimaging

# *Total body MRI scanning and therapeutic response assessments*

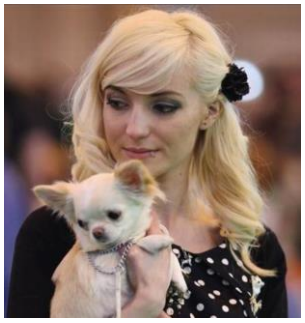


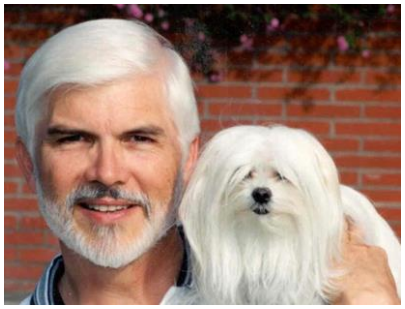
aspectimaging

ALCL-PDT Day 0 | ALCL-PDT day 14 | ALCL-PDT day 14 treated

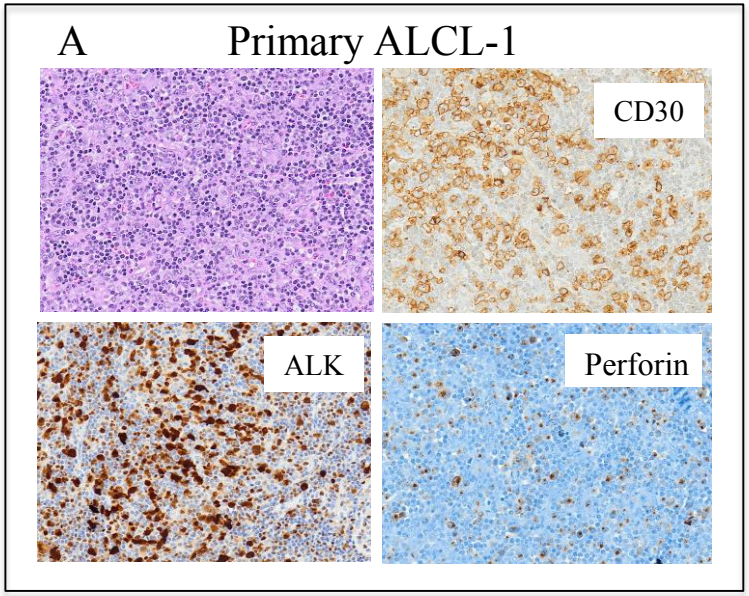


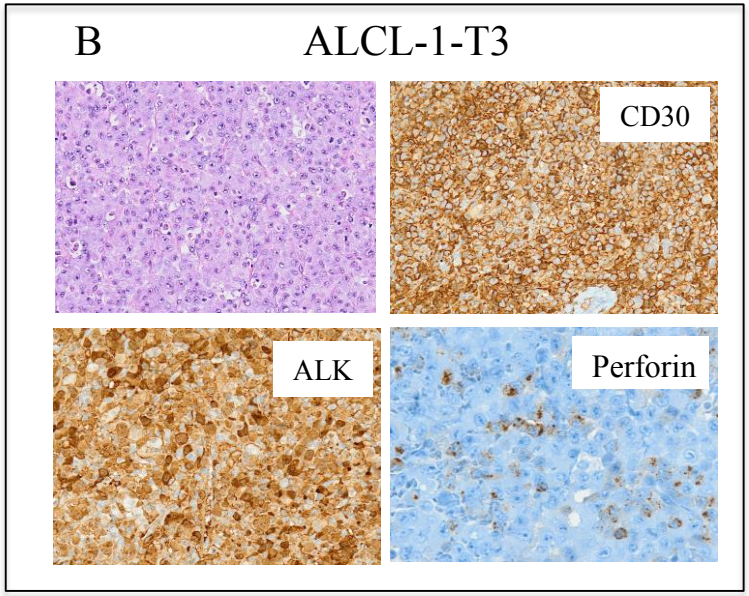
# *Do PDTX fully recapitulate their corresponding primary lesions*

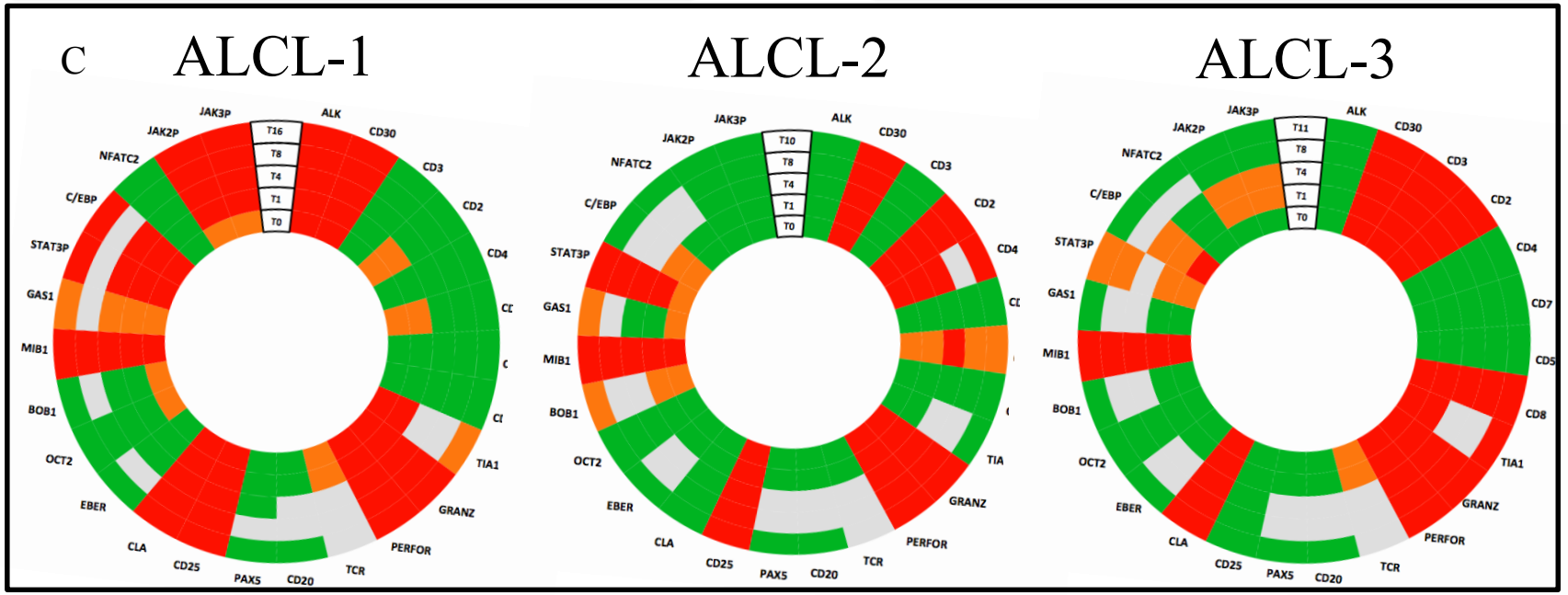




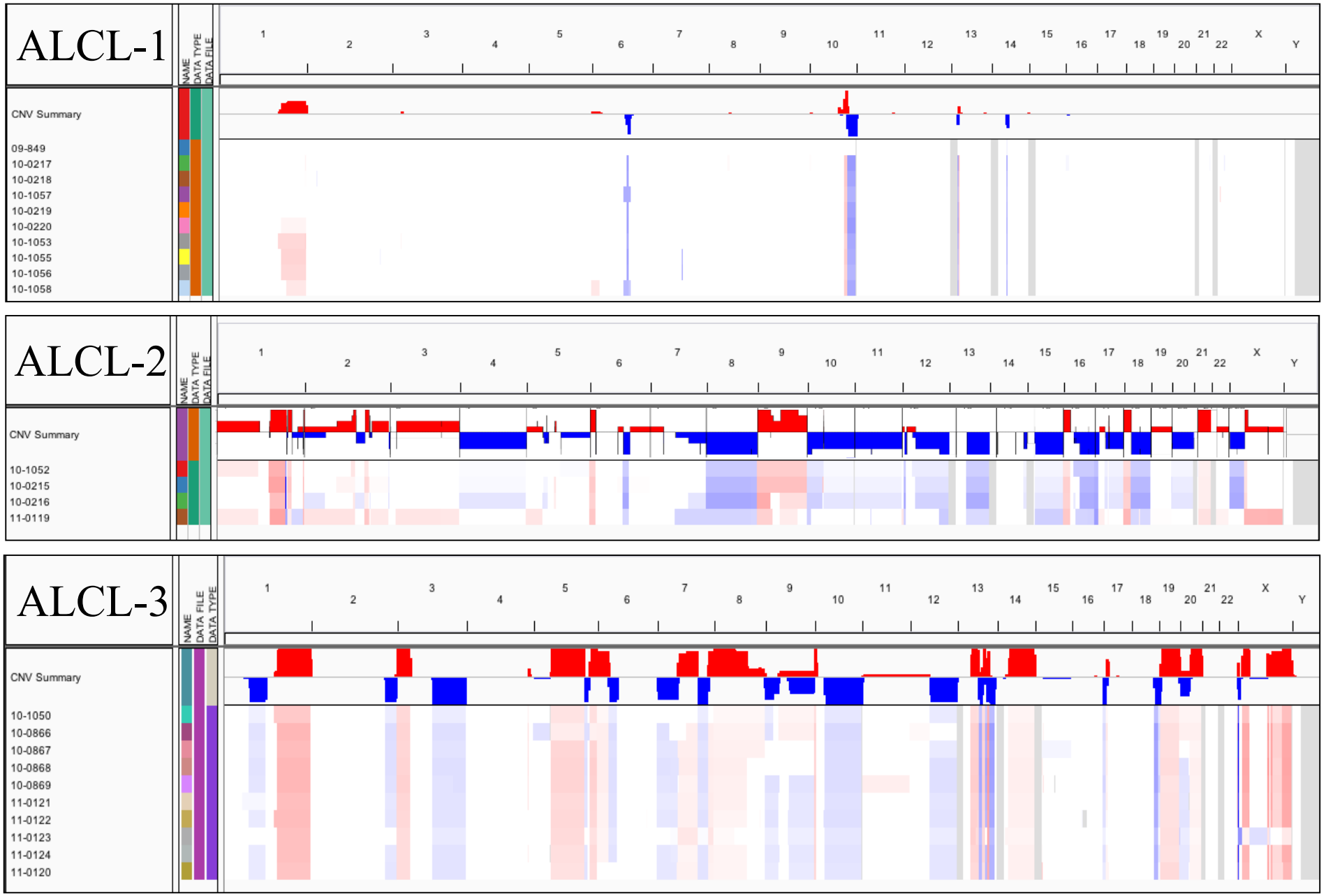
# Primary ALCL and matched ALCL tumorgrafts display identical immuno-profiles



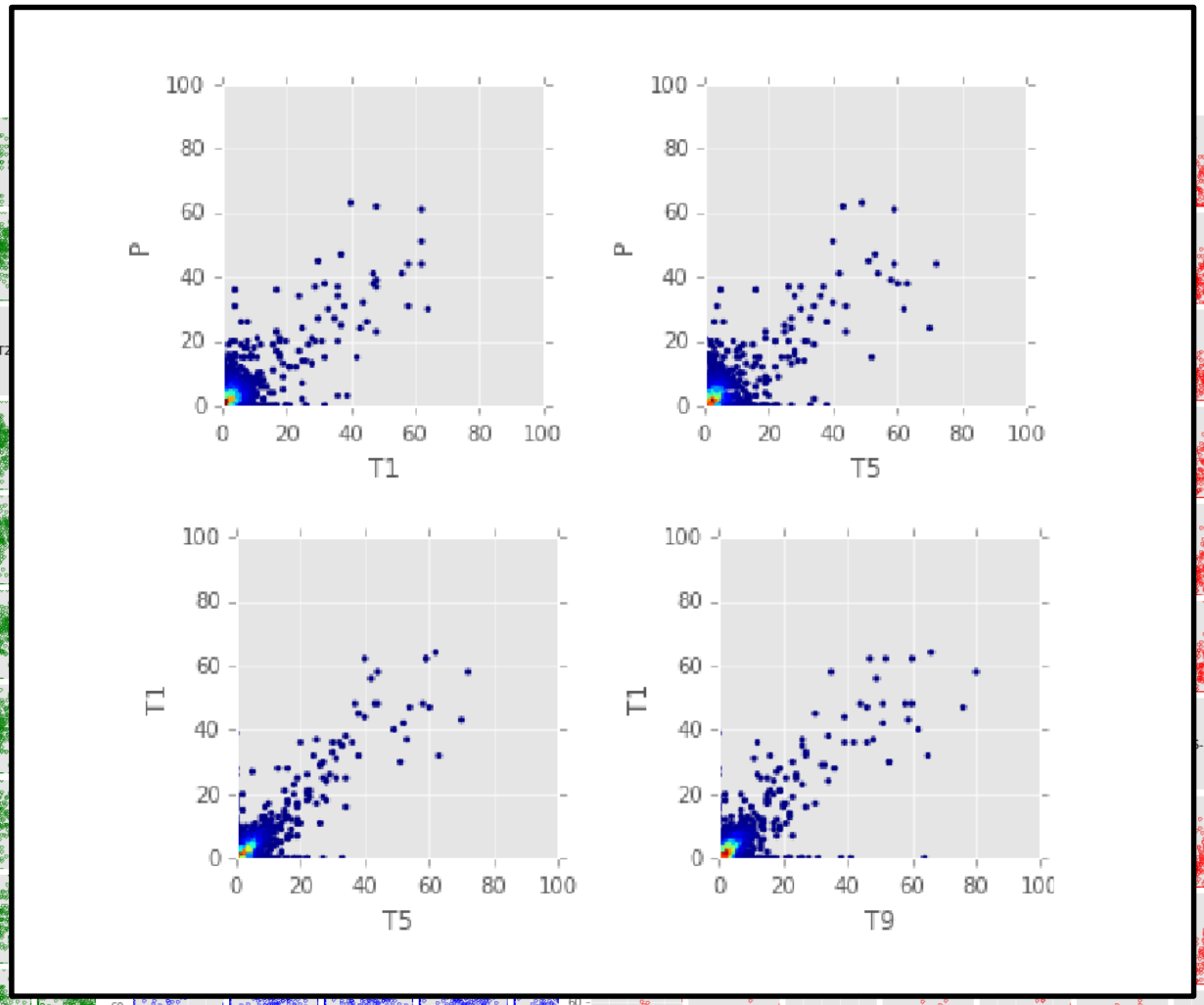



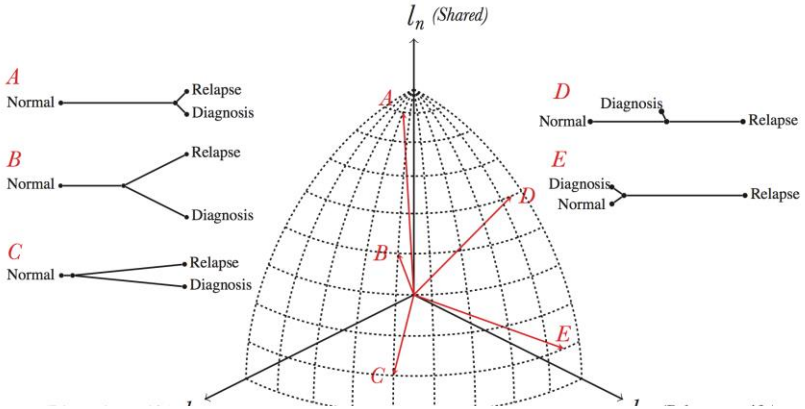
# SNP array identify analogous patient genomic defects in primary and corresponding tumorgrafts



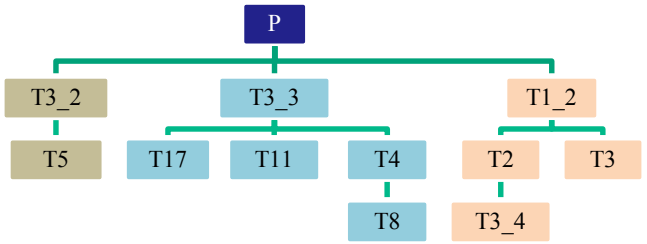
# *Does clonal evolution take place in NSG models?*

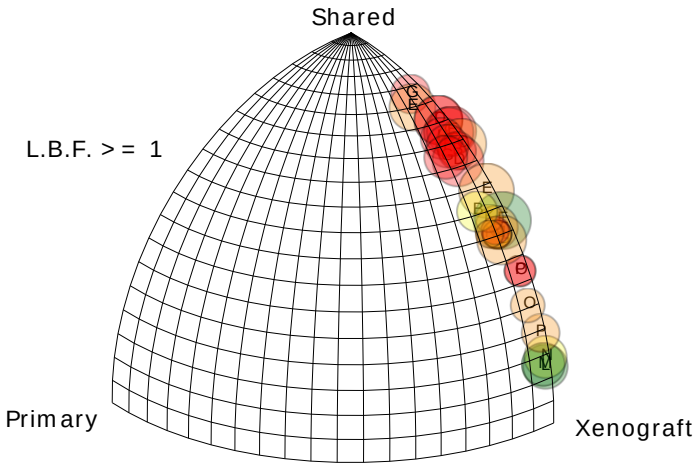


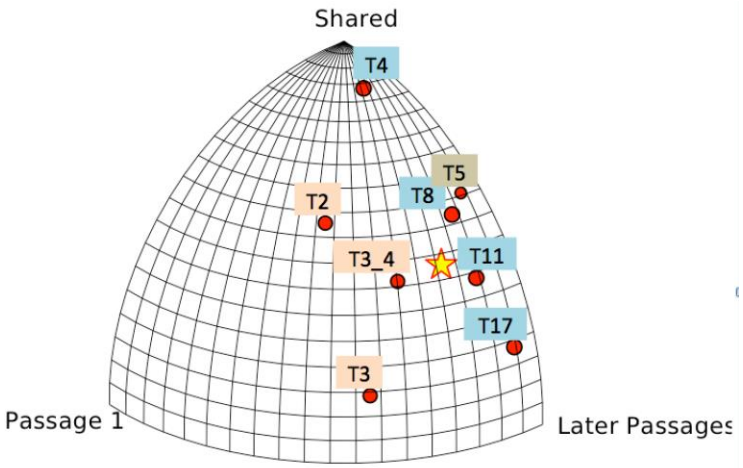
# *Evolutionary modes in PΣ3.*




ALCL-3-PDT

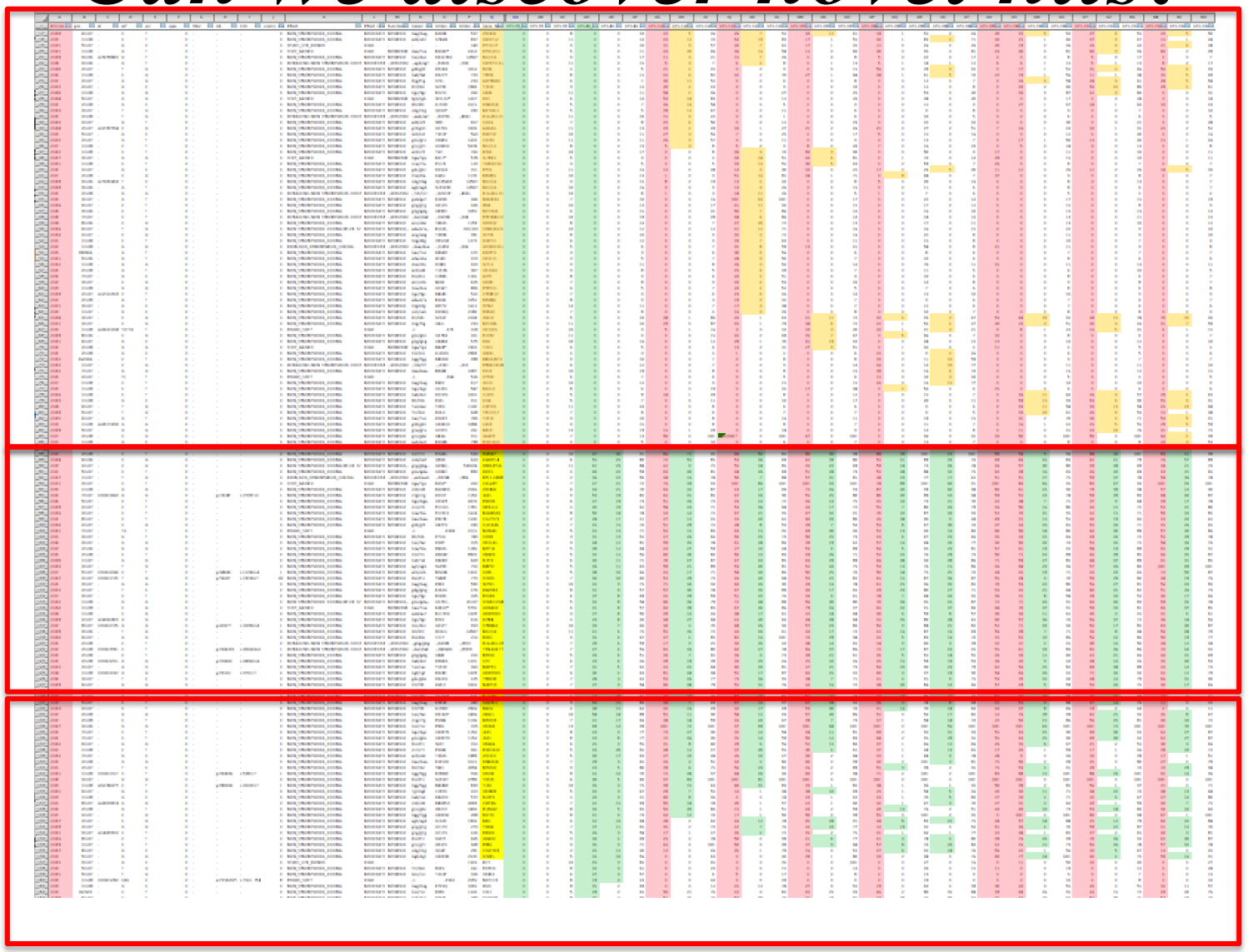


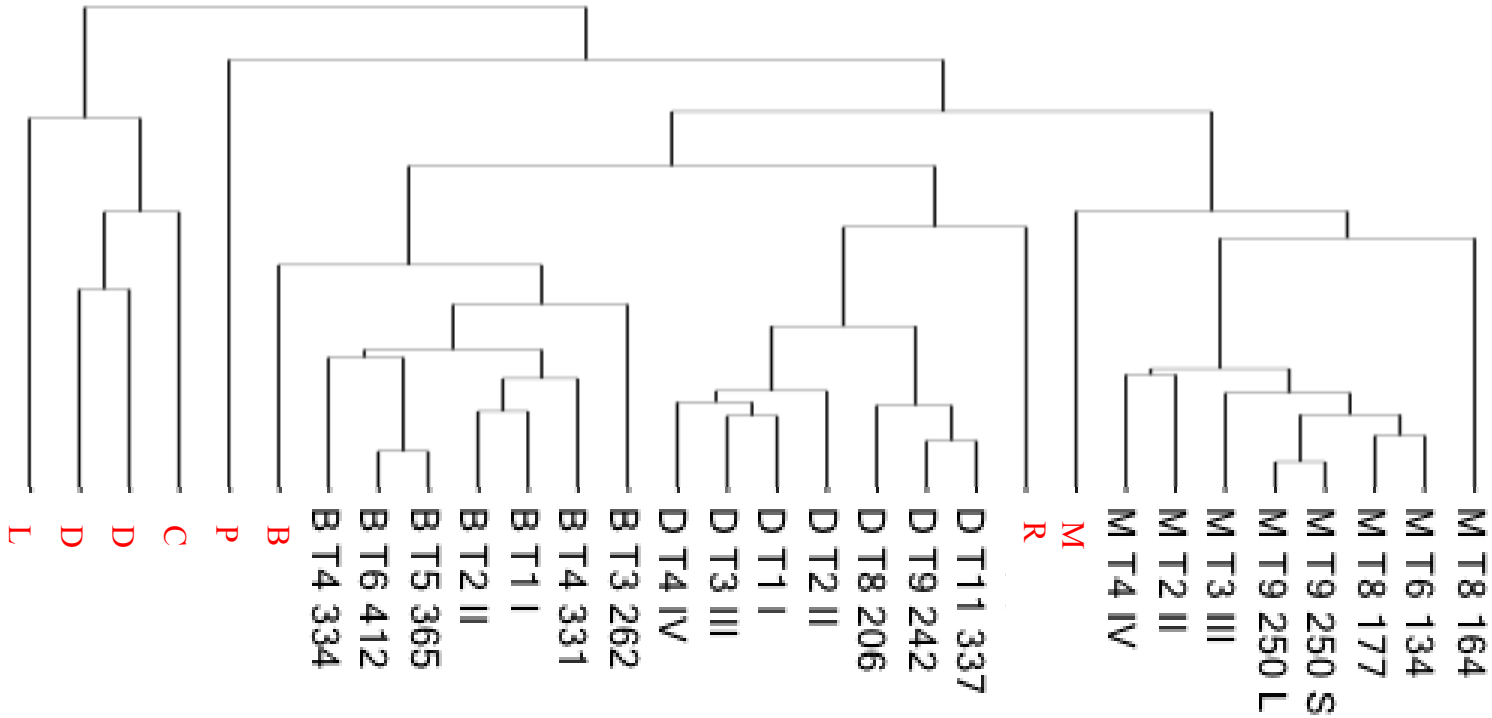




**Zairis S et al:Moduli spaces of phylogenetic trees describing tumor evolutionary patterns. In press**

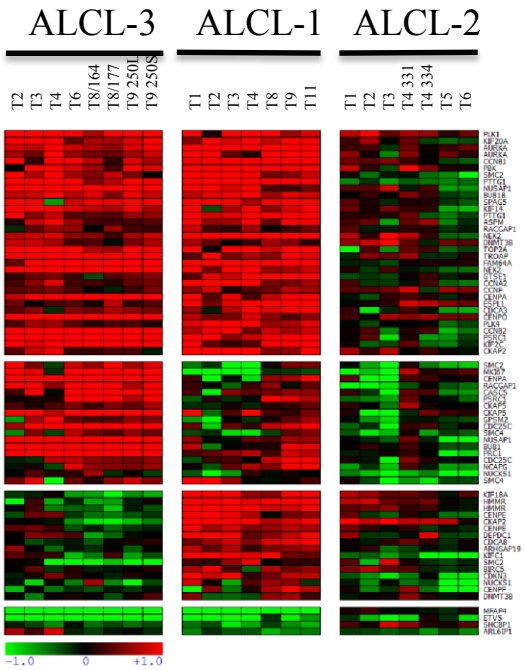
# *Can we discover novel hits?*

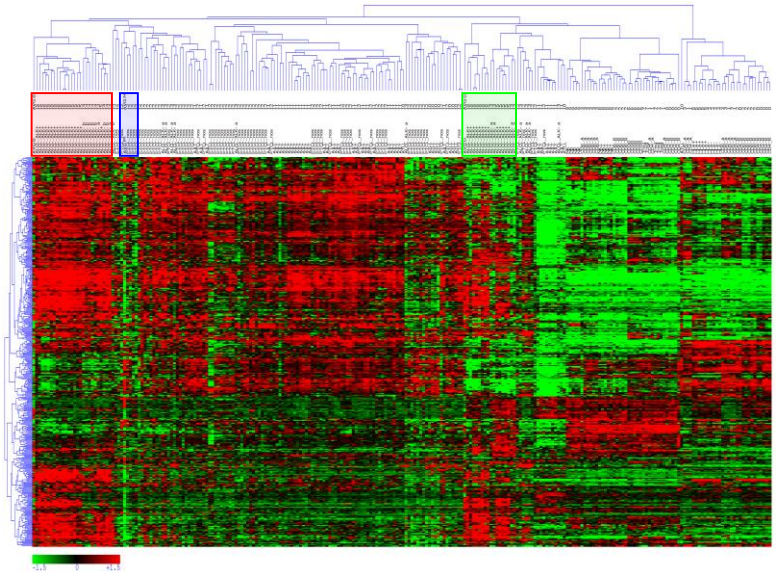


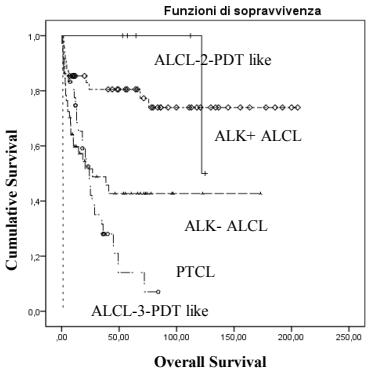
# Gene expression profiling shows unique signature for each individual tumorgraft ALCL line



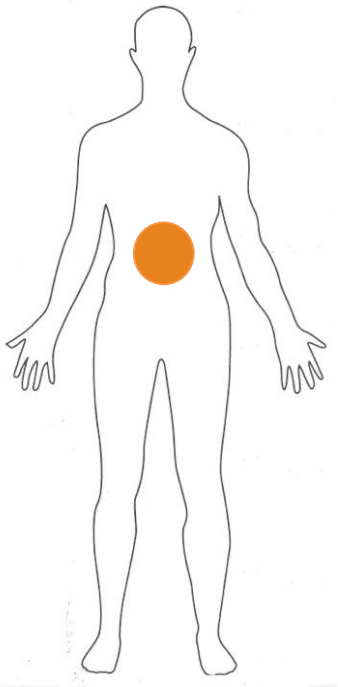
# *Gene expression signatures of ALCL-PDT identified unique subsets among PTCL patients*

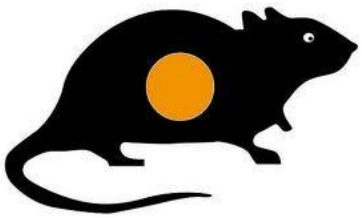


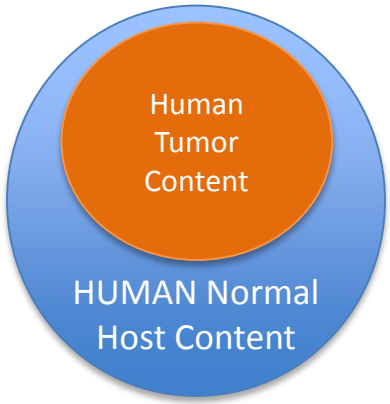



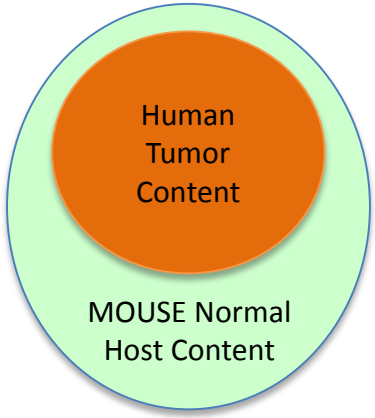


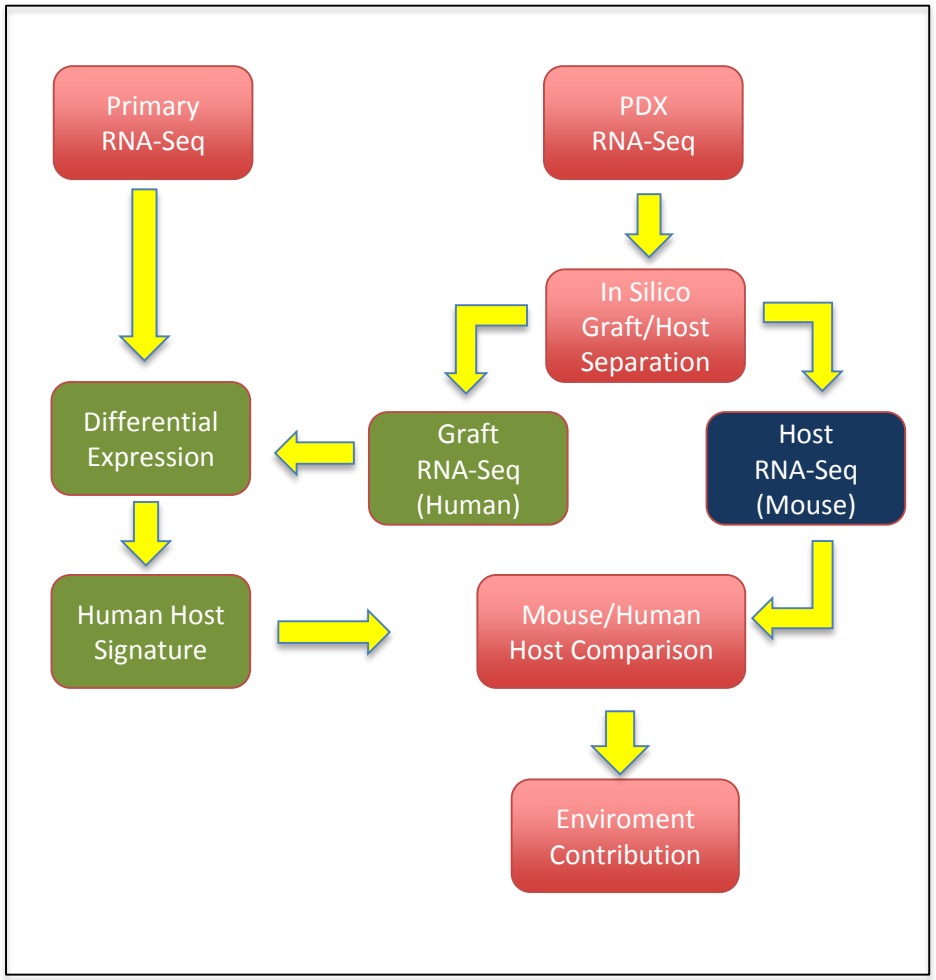
# Host-lymphoma Interactions

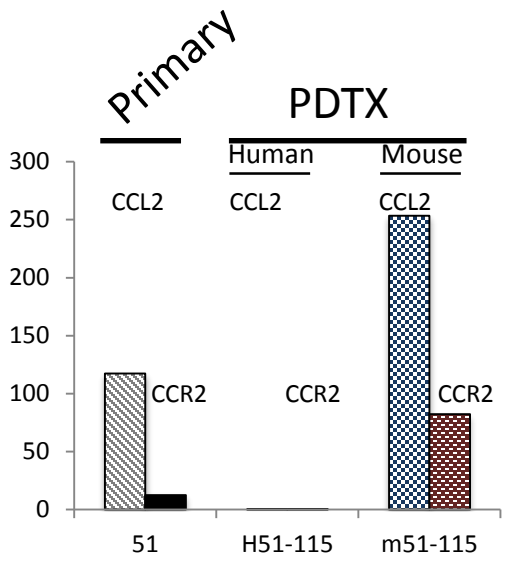


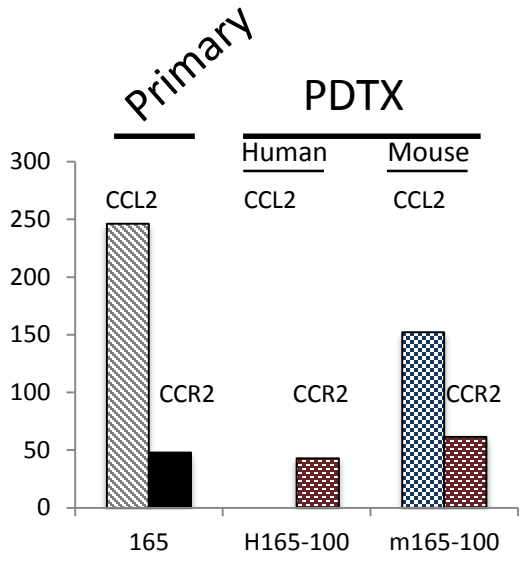


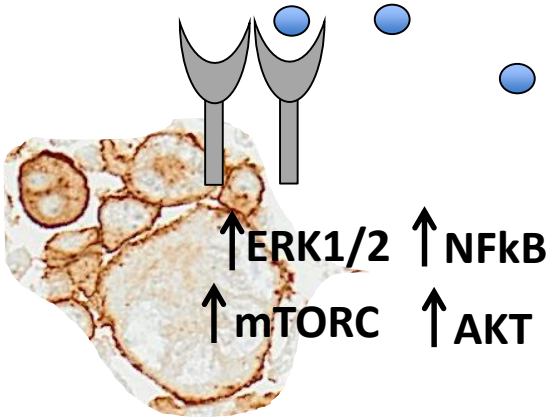


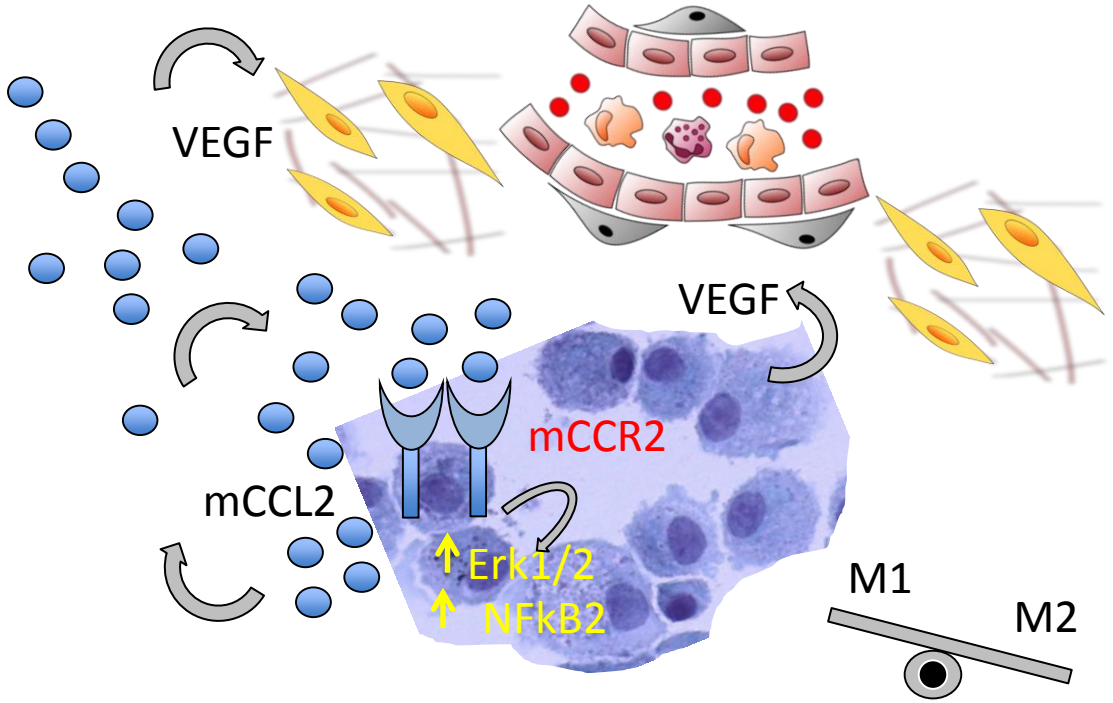




Primary
PDTX
Human
Mouse
300
250
200
150
100
50
0
CCL2
CCL2
CCL2
CCR2
CCR2
CCR2
51
H51-115
m51-115


Primary
PDTX
Human
Mouse
300
250
200
150
100
50
0
CCL2
CCL2
CCL2
CCR2
CCR2
CCR2
165
H165-100
m165-100

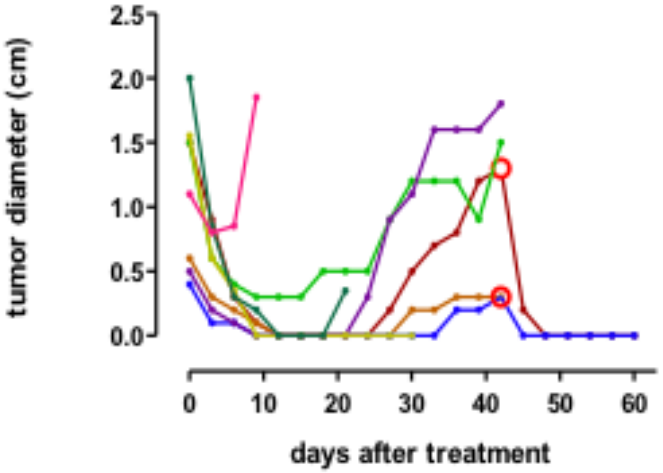

ERK1/2
NFkB
mTORC
AKT


Migration

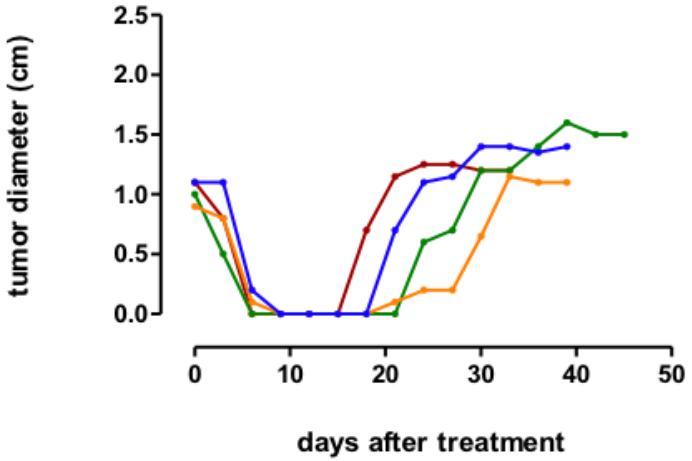

VEGF
VEGF
mCCR2
mCCL2
Erk1/2
NFkB2
M1
M2

# *NSG tumorgrafts respond to conventional treatments as matched primary ALCL*

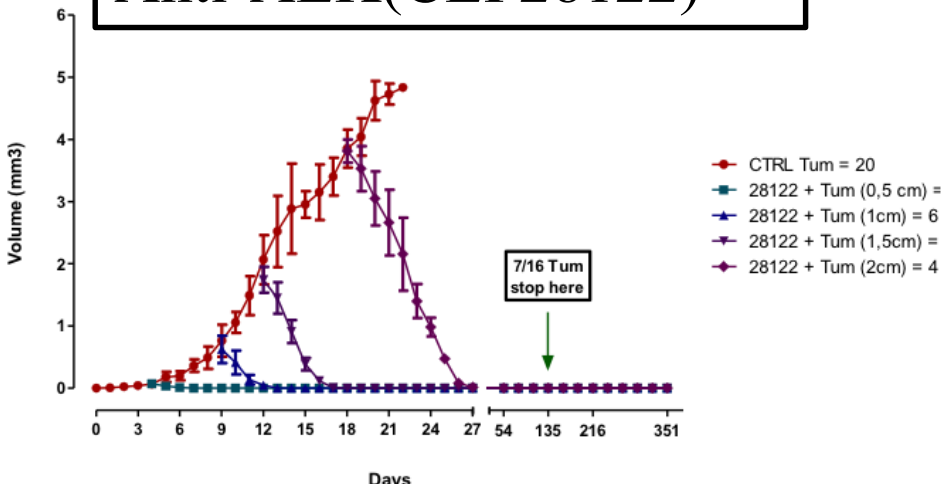
1th line CHOP




2nd line CHOP

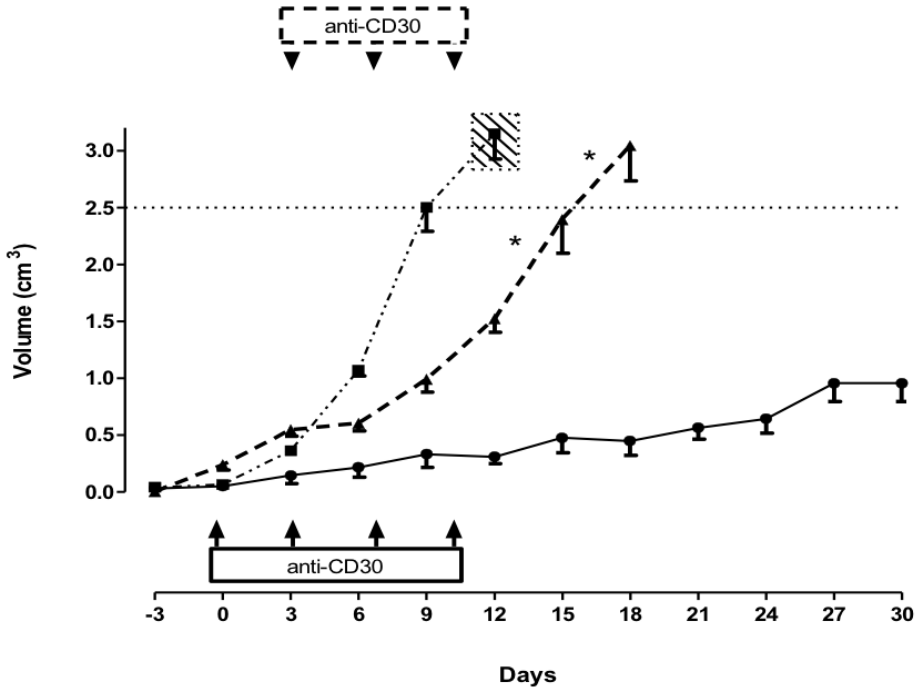



Anti-ALK(CEP28122)

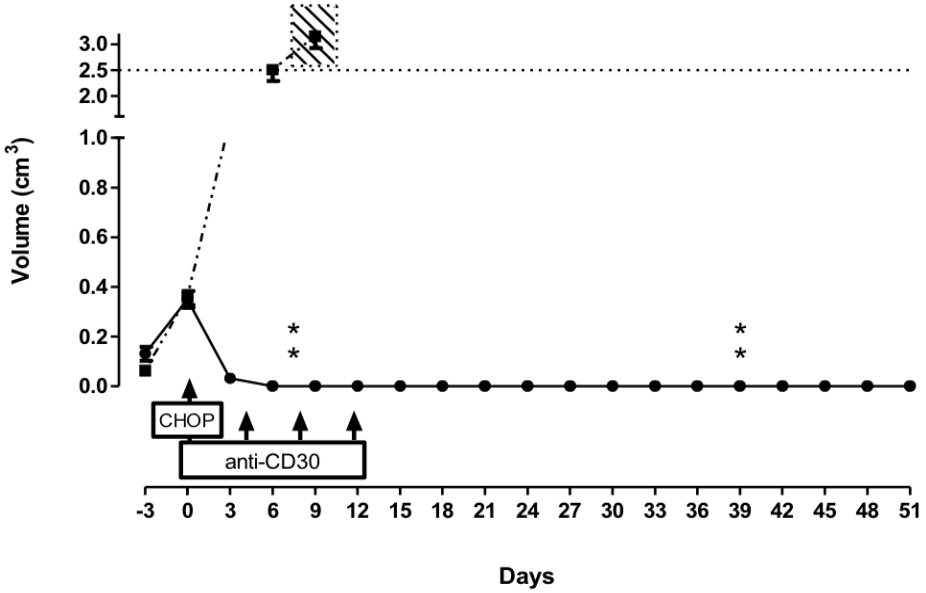



ALCL-1

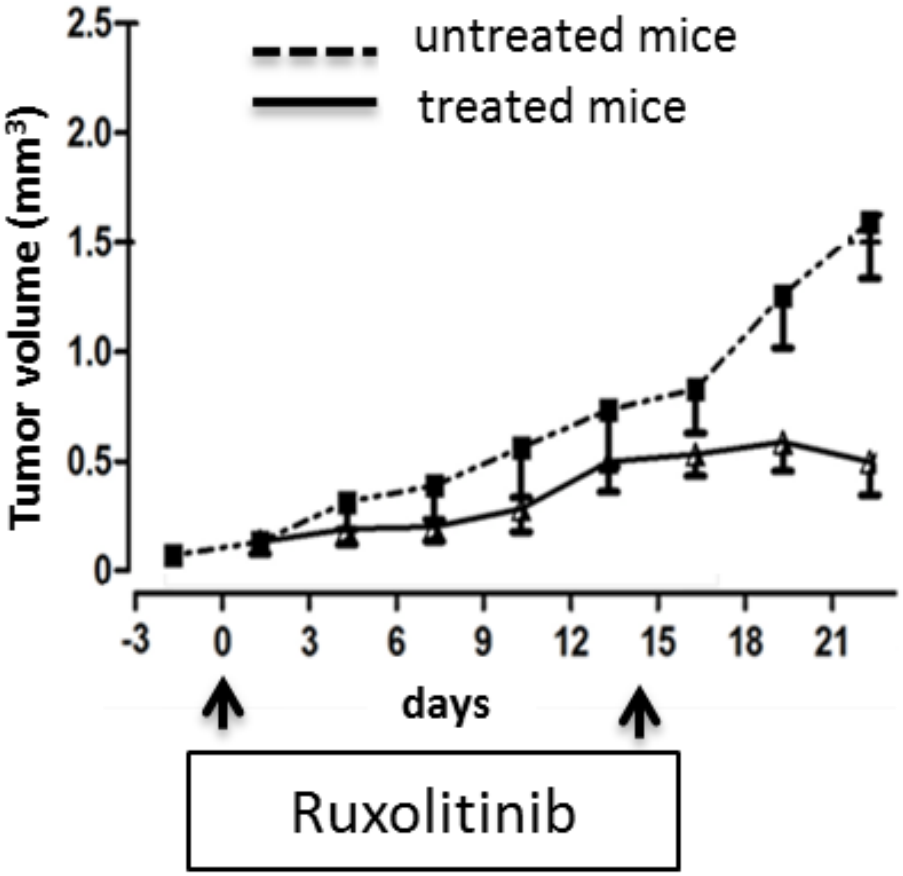
# The efficacy of SGN-35 is related on the tumor burden



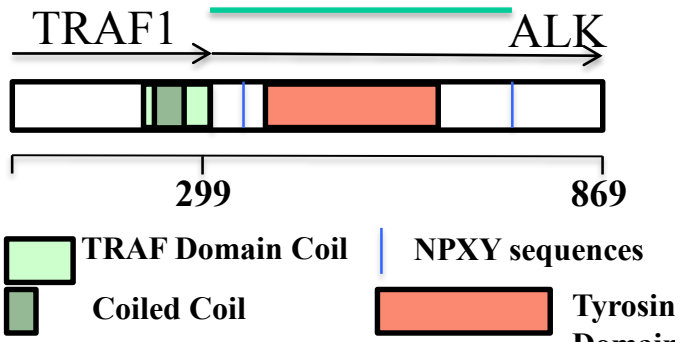

ALCL-1

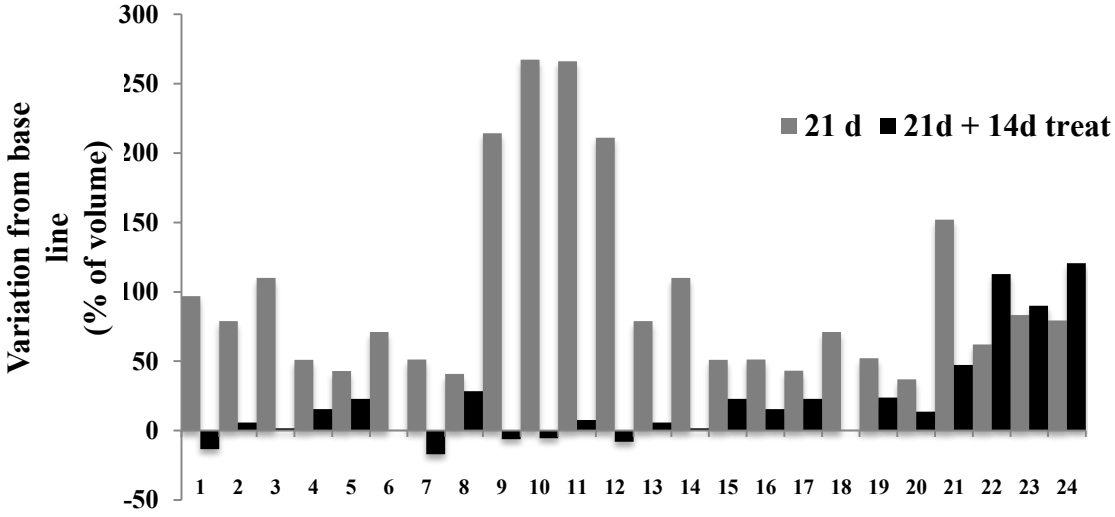


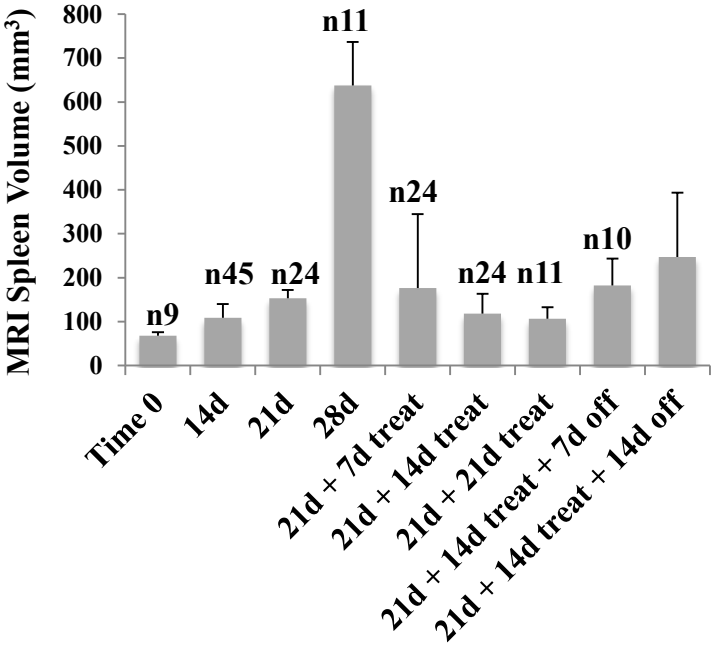
# *Anti JAK1/2 inhibitors represent an alternative approach for pSTAT3+ ALK- ALCL*

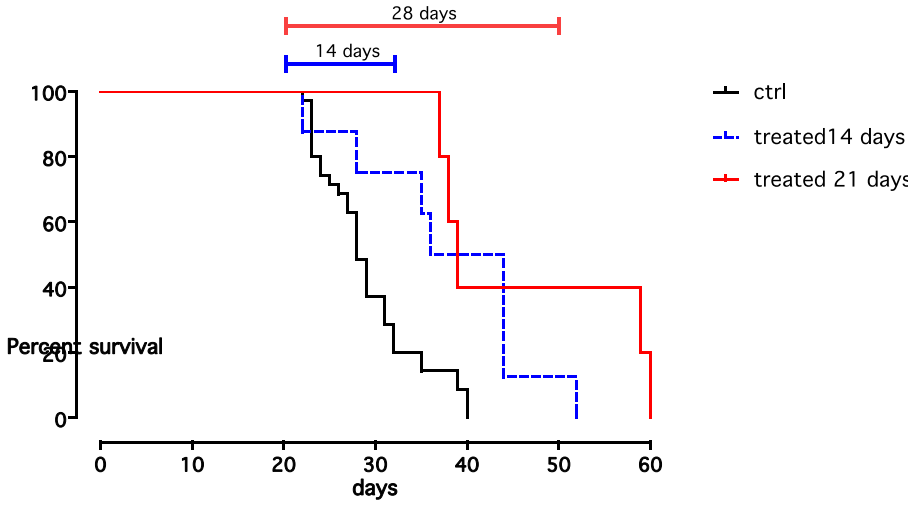


# *Treatment of ALK+ ALCL PDXT predicts clinical responses*



## *Conclusions: hurtles and problems*

- Low grade lymphoma do not successful implant
- Time of engraftment can be exceedingly long
- Many lymphoma require host support
- The relationship with the immune system is hardly reproducible in human reconstituted mice
- PDTX do not propagate successfully *in vitro*

# *Conclusions: advantages and benefits*

- Lymphoma derived PDT represent novel and powerful tools for investigating high-grade/end stage processes
- Humanized NSG mice should facilitate the creation of broad PDT libraries
- Lymphoma derived PDT need extensive genetic and functional analyses
- Molecularly annotated PDT are powerful models for testing new compounds/therapeutic strategies which can be provided to selected cohorts of cancer patients.

# Acknowledgements


**WCMC**

Fabrizio Tabbo'

Maria Todaro

Ramona Crescenzo

Marcello Gaudiano

Michela Boi

Leandro Cerchietti

**CeRMS**

Roberto Piva

Cristina Abele

Rodolfo Marchiorlatti

Katia Messana

Indira Landra

Nicoletta Chiesa

**Columbia University**

Raul Rabadan

Francesco Abate

Sakellarios Zairis

**Policlinico di Milano**

**Antonino Neri**

Luca Agnelli

**Hugef, Torino**

**Sivia Deaglio**

Tiziana Vaisitti

**IOSI**

**Francesco Bertoni**

Michela Boi

Ivo Kwee

**IRCC-Candiolo**

**Enzo Medico**

Barbara Martinoglio

**The European T-cell Lymphoma Study Group**

**Genetics-driven targeted management of lymphoid malignancies**

**AIRC 5x1000**