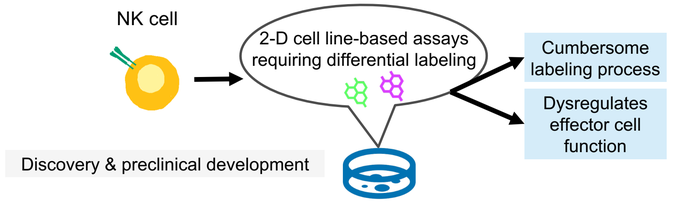
Deep learning-enabled dynamic infiltration and response to NK therapies in solid tumor organoids

Luca Lonini[1], Sonal Khare[1], Timothy D. Lopez[1], Stanislaw Szydlo[1], Michael Streit[1], Olga Karginova[1], Mary Flaherty[1], Ameen Salahudeen[2], Andre Kunert[3], Anna Oja[3], Kate Sasser[1], Martin C. Stumpe[1], Chi-Sing Ho[1]

[1]Tempus AI, Chicago, IL, USA; [2]University of Illinois Chicago, Chicago, IL, USA; [3]Sanofi R&D Oncology Research, Kiadis BV, a Sanofi company, Amsterdam, The Netherlands

Abstract #2319

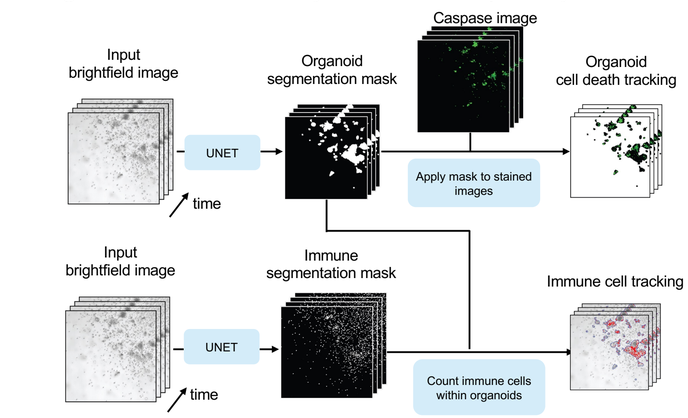
INTRODUCTION



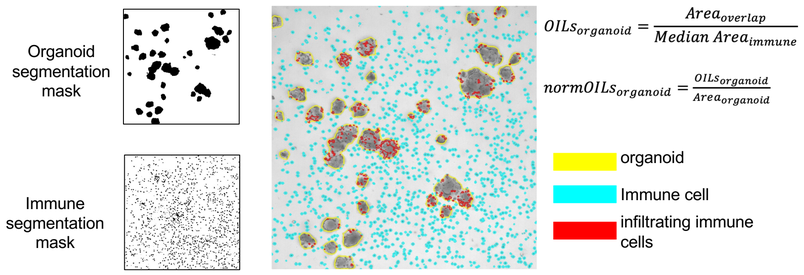
- Conventional approaches to preclinical development of cellular therapies face challenges with fluorescent labeling
- We record multi-day time-lapse confocal microscopy images of 30 patient-derived tumor organoid (TO) lines co-cultured with NK cells at increasing concentrations, and use machine vision to measure TO-specific responses in a label-free manner

METHODS

Tumor and immune cells are segmented from the brightfield channel by 2 pre-trained U-Net networks. Segmentation masks are registered with a vital dye channel to quantify TO cell death.



Immune infiltration density (Organoid-Infiltrating-Lymphocytes, aka **OILs**) is measured from the segmentation masks and correlated with TO vital dye (Caspase 3/7) at each time point.

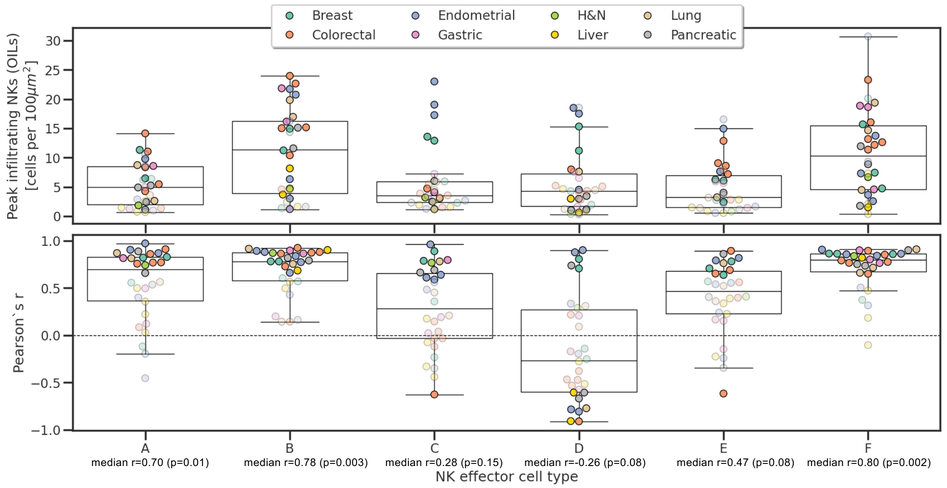


SUMMARY

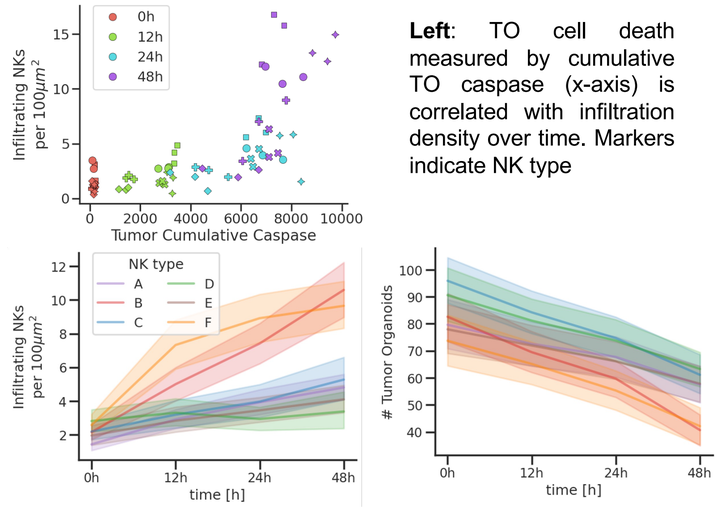
- We present a **highly scalable, label-free solution to quantify immune cell activation over time**, including infiltration, migration and co-localization dynamics, providing insights into the pharmacokinetics and the activation mechanisms for specific immune therapies.
- This approach enables **high throughput screening of candidate immunotherapies** across dozens to hundreds of unique patient-derived TO-models, thereby facilitating **targeted precision therapy**.

RESULTS

We found that the density of infiltrating immune cells (OILs) was highly correlated with TO death, as quantified by fluorescence intensity of caspase 3/7 over time. Differential infiltration dynamics are observed across TOs and immune cell lines, as peak infiltration density is affected by co-culture time, TO line, and NK cell type. Furthermore, OILs are correlated with production of effector molecules (granzyme B and IFN-γ). These findings highlight that the segmentation models can measure varying degrees of infiltration and activation across different types of effector cells and TO models.

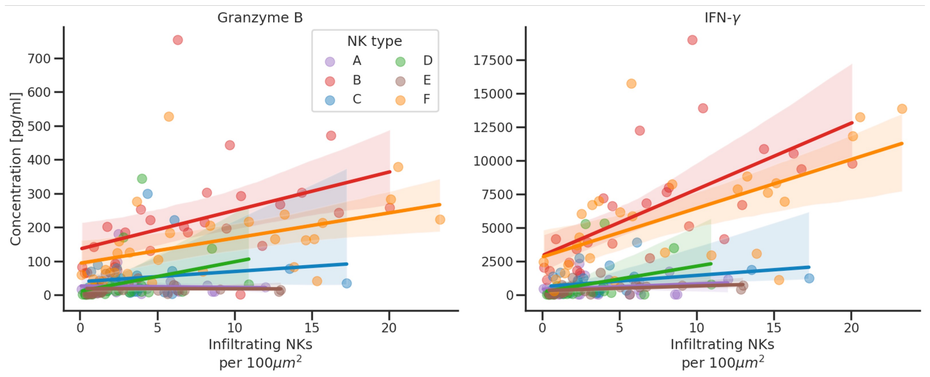


Top: NK peak infiltration density across cancer and effector cell types for a 1:6 TO-effector cell concentration. The y-axis values indicates the peak density over time. **Bottom**: Pearson correlation values between cumulative TO caspase and OILs across experiments. Transparent markers indicate non-significant (p>0.05) correlations.



Left: TO cell death measured by cumulative TO caspase (x-axis) is correlated with infiltration density over time. Markers indicate NK type

Left: NK infiltration density increases over time and is affected by the NK effector cell type (A-F). **Right**: NK therapies with increased infiltration (B and F) lead to more tumor killing, as indicated by the drop in the number of TOs in a well.



Infiltration density at 24h is correlated with the production of granzyme-B (**left**) and interferon gamma (IFN-γ, **right**) across NK therapies. Therapies with higher infiltration rates and killing rates (B and F) similarly show stronger correlations

	Pearson's r (p-value) OILs - Effector molecule concentration	
NK Type	**Granzyme B**	**IFN-γ**
A	0.18 (p=0.11)	**0.38** (p=0.004)
B	**0.59** (p<0.001)	**0.75** (p<0.001)
C	**0.34** (p<0.001)	**0.50** (p<0.001)
D	**0.35** (p<0.001)	**0.35** (p=0.0056)
E	**0.25** (p=0.02)	**0.51** (p<0.001)
F	**0.65** (p<0.001)	**0.73** (p<0.001)

Acknowledgments: We thank Dana DeSantis from the Tempus Scientific Communications Team for poster review.

Correspondence: luca.lonini@tempus.com