Abstract #603

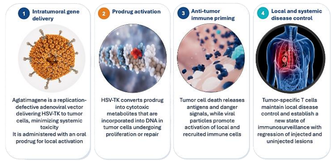
Multi-omics analyses reveal intratumoral and systemic immune activation to aglatimagene besadenovec + valacyclovir related to improved survival in patients with progressive NSCLC despite ICI treatment

Caroline Duault*, PharmD, PhD (1); Giuseppe Barisano*, MD, PhD (1); Emily J. Mastej, PhD (1); Davide G. Franchina, PhD (1); Christine Camacho Fullaway, BS (1); Alex Kong, MSc (1); Jolene S. Ranek, PhD (1); Sangeeta Kowli, PhD (1); Angelina Spence, BS (1); Vamseelatha Pakir (1); Mina Pichavant (1); Hui Xie (2) ; Tia Rizakos (2) ; Kai Nie (2) ; Seunghee Kim-Schulze, PhD (2) ; Diane Del Valle, PhD (2) ; Sacha Gnjatic, PhD (2) ; Charu Aggarwal, MD (3); Daniel Sterman, MD (4); Jessica Dwyer (5); Andrea Manzanera, MD (5); Garrett Nichols, MD (5); Paul P. Tak, MD, PhD (5); Francesca Barone, MD, PhD (5); Vivek Charu, MD, PhD (1); Sean Bendall, PhD (1); Holden Maecker, PhD (1)
* Co-first authors. (1) Stanford University School of Medicine, Stanford, CA, (2) Icahn School of Medicine at Mt Sinai, New York, NY, (3) University of Pennsylvania's Perelman School of Medicine, Philadelphia, PA, (4) NYU Langone Health, New York, NY, (5) Candel Therapeutics, Needham, MA

Contact: cduault@stanford.edu

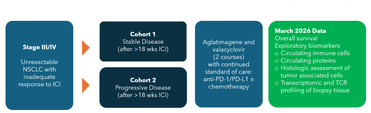
Background

- Patients with unresectable stage III/IV non-small cell lung cancer (NSCLC) progressing despite immune checkpoint inhibitors (ICI) have limited treatment options [1].
- Aglatimagene besadenovec (CAN-2409) is a replication-defective adenoviral vector encoding herpes simplex virus thymidine kinase. Intratumoral administration with valacyclovir induces immunogenic tumor cell death and systemic immune activation, supporting combination with continued ICI [1].
- The open-label phase 2a trial (NCT04495153) evaluated two intratumoral injections plus valacyclovir with continued ICI. Survival findings support further investigation, but historical comparisons cannot establish treatment benefit [1].
- Here, we integrate longitudinal tumor and blood multi-omics profiling to characterize intratumoral and systemic immune changes and explore molecular and cellular correlates of overall survival.



LuTK02 clinical study

Phase 2a clinical trial of aglatimagene + continued ICI in stage III/IV NSCLC patients with an inadequate response to ICI

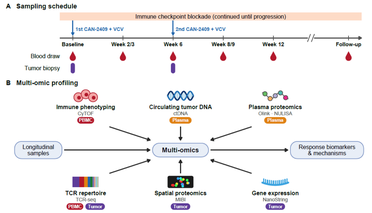


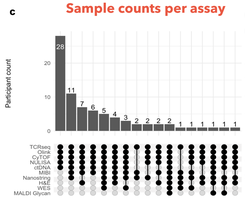
Patients were enrolled into two cohorts based on their prior response to ICI: those with stable disease during ICI treatment (Cohort 1) and those with disease progression despite continued ICI (Cohort 2), as documented by serial CT scans in both cohorts. More patients were enrolled in Cohort 2, reflecting the greater unmet clinical need in this group.

Survival - cohort 2

In evaluable/per-protocol cohort 2 patients (n=41), mOS from first injection was 21.5 months (95% CI 14.3-31.2; median follow-up 32.4 months)

Methods

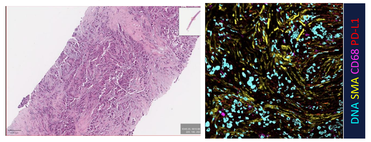




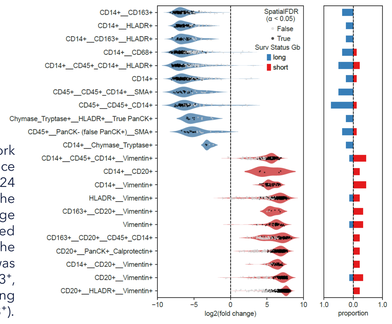
(A) Treatment and sampling schedule. (B) Multi-omics analysis performed on patients from cohort 2 evaluable for efficacy (n = 41). (C) Assays by sample type. Right: participants per combination of assays (filled dots = assay available). Most patients (n = 28) have blood-based assays only; fewer have paired tumor data. Multiplicity correction was assay- and analysis-specific; no global adjustment was performed across modalities.

Baseline spatial immune niches differ between patients with OS >24 versus <24 months

Multiplexed ion beam imaging (MIBI)



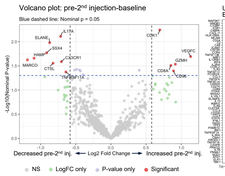
QUantitative InterCellular niCHe Enrichment (QUICHE) analysis



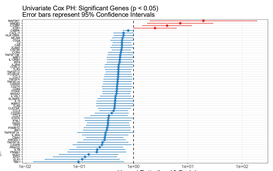
QUICHE analysis of baseline biopsies (n = 23). QUICHE is a spatial framework that identifies niches (groups of neighboring cell types) whose abundance differs between conditions (short survival: <24 months, n=9 ; long survival: >24 months, n=8), with a spatial false discovery rate (FDR) accounting for the dependence between neighboring cells. Violins show the log2 fold change distribution per niche (negative = enriched in long survival, positive = enriched in short survival); dark dots indicate spatial FDR < 0.05. Bars show the proportion of corresponding niches found in each condition. Long survival was associated with niches made of monocytes/macrophages (CD14⁺, CD163⁺, CD68⁺, HLA-DR⁺), whereas short survival was associated with niches combining B cells (CD20⁺) or stromal cells (Vimentin⁺) with macrophages (CD14⁺, CD163⁺).

NanoString nCounter analysis suggests cytotoxic response activation in tumor tissue

Delta 1st injection to baseline

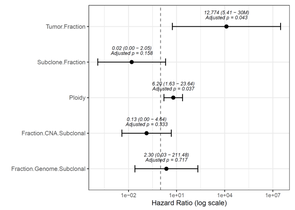


Univariate Cox proportional hazard model for OS



Increased CD8A, GZMH and CD96 suggest NK/CD8 T cell infiltration while decreased ELANE, MARCO and CTSL point to decreased neutrophils and macrophages (including TAM) signals. Higher baseline expression of genes associated with longer OS (CXCL11, CD274, IRF1, CCL4, PSMB10, HLA-DMA, ICAM1) is consistent with an IFN-γ/inflamed tumor phenotype. Exploratory analysis, only raw p < 0.05 values were reported.

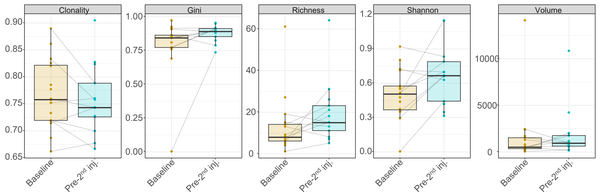
Higher baseline tumor fraction and ploidy were associated with higher mortality risk



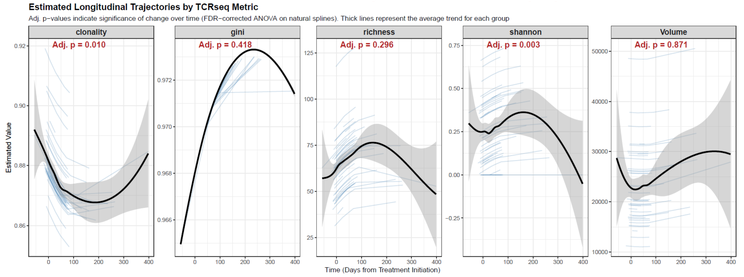
ctDNA analysis was performed on plasma with ultra-low-pass WGS. 27 baseline samples were analyzed with an exploratory univariate Cox proportional hazard model. Higher baseline ctDNA tumor fraction, a proxy for tumor burden, and higher tumor ploidy were associated with shorter OS (adjusted p = 0.043 and 0.037). Subclonal fractions showed no association (adjusted p ≥ 0.16).

TCR sequencing of tissue and peripheral blood reveals increased T cell repertoire diversity post aglatimagene besadenovec

TCR repertoire diversity increases from Baseline to pre-2nd injection in tumor tissue



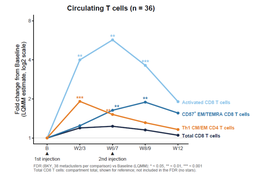
Clonality decreases and Shannon diversity increases over time in peripheral blood



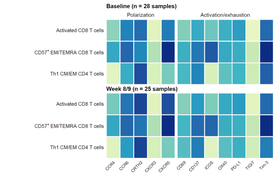
TCR sequencing was performed on 29 tissue samples from 20 patients and 179 PBMC samples from 40 patients. Gini index reflects inequality in clone sizes (0 = all clones equally abundant, 1 = a few clones dominate). Shannon index reflects both the number of clones and how evenly they are represented (higher = more diverse repertoire).
In PBMC, curves show estimated trajectories from natural-spline models: blue lines = individual patients, black line = group average, grey band = 95% CI. Aglatimagene besadenovec was associated with increased T cell repertoire diversity in both tumor tissue and peripheral blood. None of the TCRseq metrics (baseline value, current value, or rate of change) were significantly associated with overall survival in Cox models (all adjusted p ≥ 0.38).

Peripheral immune profiling shows T cell activation after aglatimagene besadenovec

Activated CD8 and Th1 CD4 T cells expand in blood after the first injection



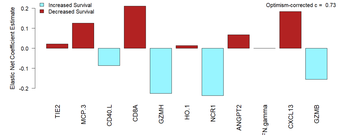
Expanding T cells express CXCR3 and TIGIT, with a stable phenotype over time



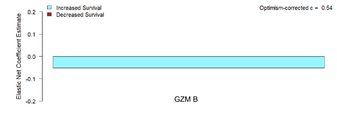
PBMC were analyzed by mass cytometry (CyTOF), and T cell subsets were defined by FlowSOM metaclustering. Left: fold change in subset abundance from Baseline, estimated by linear quantile mixed models (LQMM; n = 36 patients). Activated CD8 T cells increased up to ~5-fold, peaking just before the second injection (Week 6/7); memory subsets were defined upon expression of CCR7 and CD45RA: Naïve = CCR7⁺CD45RA⁺, Central Memory (CM) = CCR7⁺CD45RA⁻, Effector Memory (EM) = CCR7⁻CD45RA⁻, Terminally differentiated effectors (TEMRA) = CCR7⁻CD45RA⁺. Th1 CM/EM CD4 T cells peaked early (Week 2/3); CD57⁺ EM/TEMRA CD8 T cells rose from Week 6/7 to Week 8/9. Stars indicate FDR-adjusted significance vs Baseline (Benjamini-Krieger-Yekutieli, 38 metaclusters per comparison). Total CD8 T cells are shown for reference and are not included in the FDR. Right: median % of cells positive for each marker in the same three subsets at Baseline (n = 28) and Week 8/9 (n = 25), in samples with ≥ 20 events in the subset. Log10 colour scale from 0.1% to 100%. All three subsets expressed CXCR3 and TIGIT, and their marker profiles were similar at Baseline and Week 8/9.

Plasma proteomics links cytotoxic protein dynamics to OS

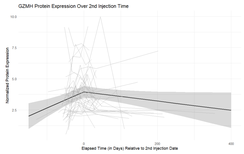
Olink: protein fold changes associated with OS



NULISA: GZMB link to a better outcome is confirmed



GZMH rises after the 1st injection



Landmark analysis included patients alive with paired baseline and post-second-injection (Week 8/9) samples; follow-up for the Cox model began at the second-injection landmark in 39 patients (23 deaths). Elastic net Cox models were fitted on the delta (landmark-baseline). Olink (81 proteins): larger increases in cytotoxic granule, NCR1 and CD40L were associated with longer OS, whereas TIE2, ANGPT2 (angiogenesis), CD8A, MCP-3, HO-1 and CXCL13 were associated with shorter OS (optimism-corrected C-index 0.73). NULISA (244 proteins): only GZMB was selected with longer OS, but with limited predictive value (C-index 0.54). GZMH was the only protein with a significant trajectory, rising before the second injection (adj. p = 0.05) and declining afterwards (adj. p = 0.04). No protein was significantly associated with OS after FDR correction (Benjamini-Hochberg).

Conclusions

- Aglatimagene treatment was associated with coordinated immune activation, including expansion of activated CD8⁺ and Th1 CD4⁺ T cells, increased peripheral TCR diversity, and increased tumor cytotoxic/NK-cell signals.
- Baseline tumor immune features and tumor burden, as well as selected on-treatment plasma protein changes, were associated with OS in exploratory analyses.
- These findings support pharmacodynamic immune engagement and identify hypotheses for prospective validation.
- These exploratory analyses were performed in a small cohort and are hypothesis-generating. No individual protein or TCR metric was significantly associated with OS after multiple-testing correction.

References and Acknowledgements

References: ***1. Aggarwal C., et al. Prolonged survival after systemic immune reactivation by aglatimagene besadenovec plus valacyclovir in ICI-refractory NSCLC. J Immunother Cancer 2026;14:e015821.***

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