Evaluation of the immune response using TCR repertoire analysis in patients with non-small cell lung cancer treated with a PDC*line cell-based cancer vaccine in combination or not with an anti-PD-1

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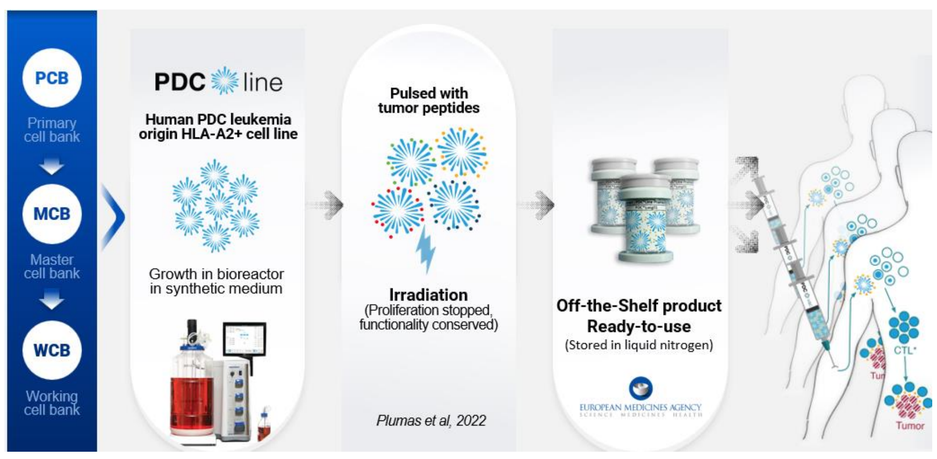
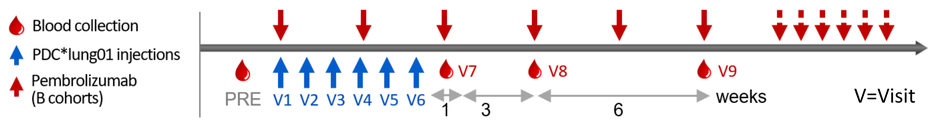
PDC*lung01
Off-the-shelf plasmacytoid dendritic cell-based product

- PDC*lung01 (IMP) is a therapeutic cancer vaccine based on an irradiated plasmacytoid dendritic cell line loaded with HLA-A*02:01-restricted peptides (NY-ESO-1, MAGE-A3, MAGE-A4, Multi-MAGE-A, MUC1, Survivin and Melan-A) able to prime and expand peptide-specific CD8+ T cells *in vitro* and *in vivo.* PDC*lung01 was shown to expand antitumor CD8+ T-cells from PBMC of patients with melanoma or NSCLC and to be synergistic with anti-Programmed Cell Death (PD)-1 (Pembrolizumab®; Charles, OncoImmunol 2020; Lenogue, Vaccines 2021; Hannani, Int. J. Mol. Sci. 2023).



PDC-LUNG-101 study design (NCT03970746)

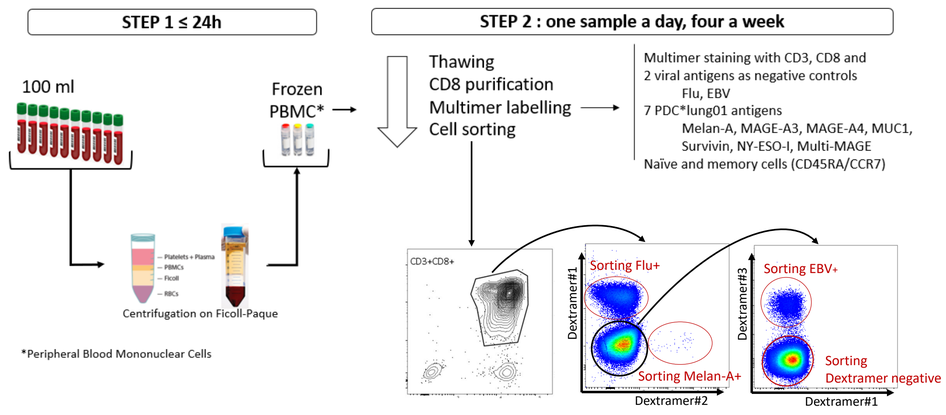
	Cohort	Arm	# of patients	NSCLC Patient Criteria	Main Objectives
Phase I	PDC*lung01 Mono	A1 Low dose	2M cells/peptide	6	• **Stage IIa/IIb/IIIa** after R0 resection • **Adjuvant chemotherapy** +/- radiotherapy	• **Safety & tolerability** • **Immune activity**
		A2 High dose	20M cells/peptide	10		
Phase II	Anti-PD-1 + PDC*lung01	B1 Low dose	2M cells/peptide + anti-PD-1	6	• **Stage IV** starting **anti-PD-1** as **first-line (TPS≥50%)**	• **Dose range** • **Clinical activity:** - **Overall Response Rate (ORR)** - **Median Progression Free Survival (mPFS)** - **Disease Control Rate (DCR)**
		B2 High dose	20M cells/peptide + anti-PD-1	45		



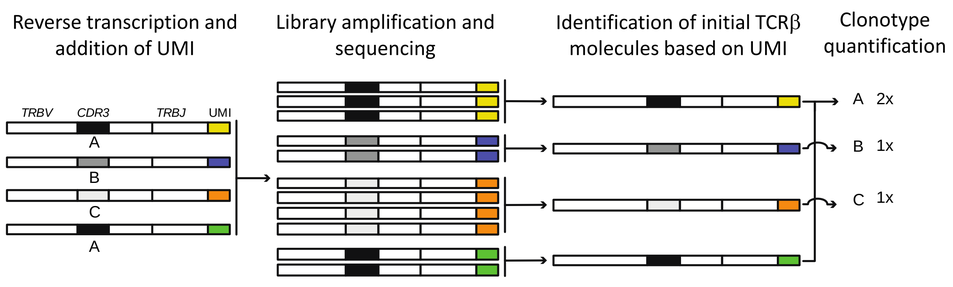
- PDC*lung01 administration schedule: IV+SC every week (6 times)
- Anti-PD-1 administration schedule: IV, every 3 weeks (until progression)

Immunomonitoring assays and qTCRseq

- Leukocyte count and peptide-specific CD8+ T were monitored in the bloodstream at different times before and after vaccination. The different populations of interest are sorted based on dextramer staining.



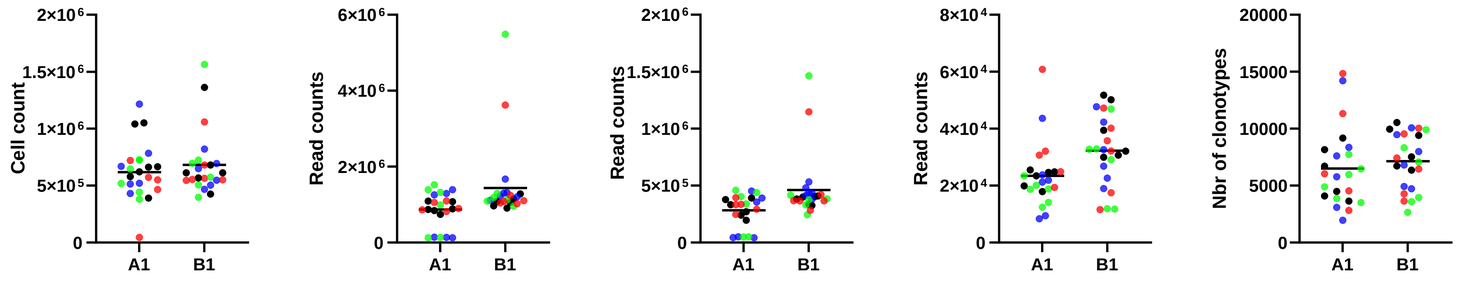
- Quantitative TCR sequencing (qTCRseq): RNA was extracted from sorted T CD8+ cells and reverse transcribed to cDNA with the addition of a Unique Molecular Identifier (UMI) on each TCRβ molecules. The UMI allows to correct for amplification biases during library preparation or sequence errors introduced during the complete sequencing process and to obtain the absolute quantification of molecules sequenced.



- qTCRseq was performed on samples from patients of cohort A1 and B1

TCR repertoire analysis from dextramer-negative CD8+ cell population

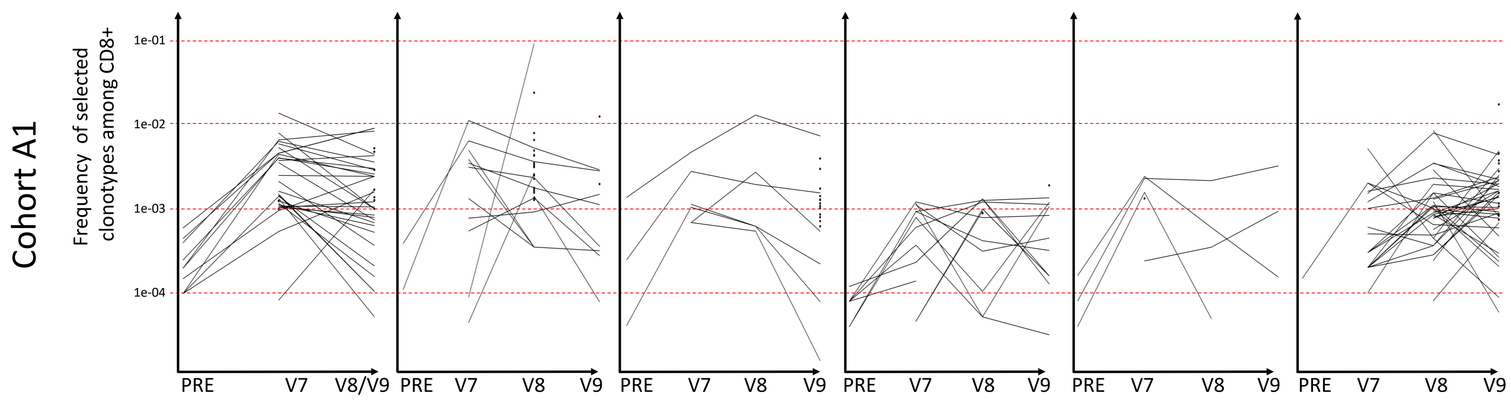
- **qTCRseq metrics overview : screening – V7 – V8 – V9**



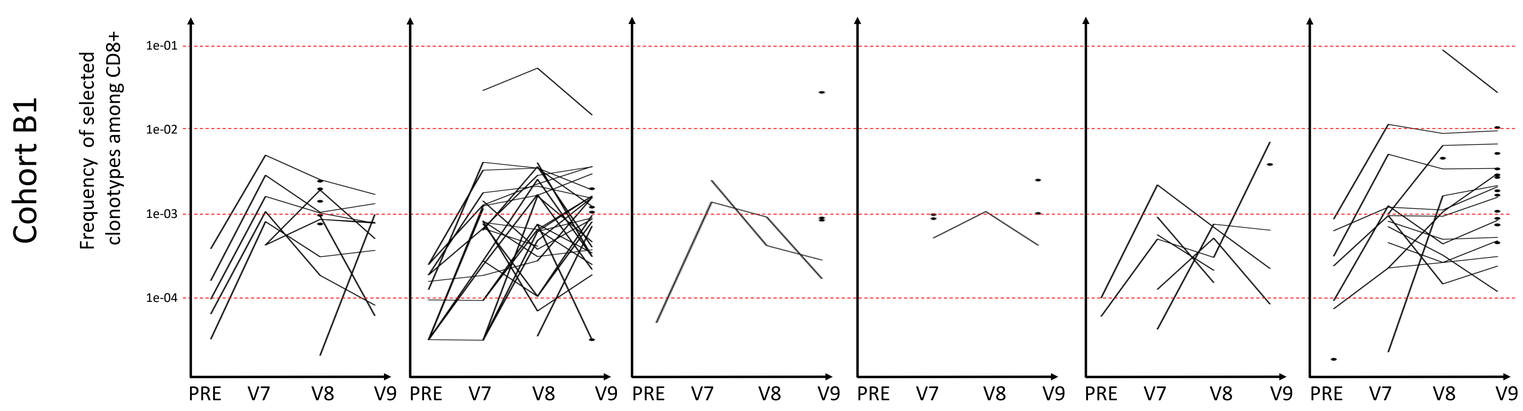
- **Analysis strategy to identify clonotypes that increase in the blood after vaccination (selected clonotypes):**

qTCR-seq data from dextramer negative CD8+ samples from one patient → Pairwise comparison: Screening vs V7, Screening vs V8, Screening vs V9 → Selection of clonotypes with at least 10-fold increase post-treatment and $p<10^{-6}$ → Chronological monitoring of each selected clonotype identified at one timepoint in the other timepoints

- **Frequencies of selected clonotypes within T CD8+ cell population:** lines correspond to frequency variation of selected clonotypes detected at more than one timepoint during monitoring, and dots represent clonotypes identified at a single timepoint.



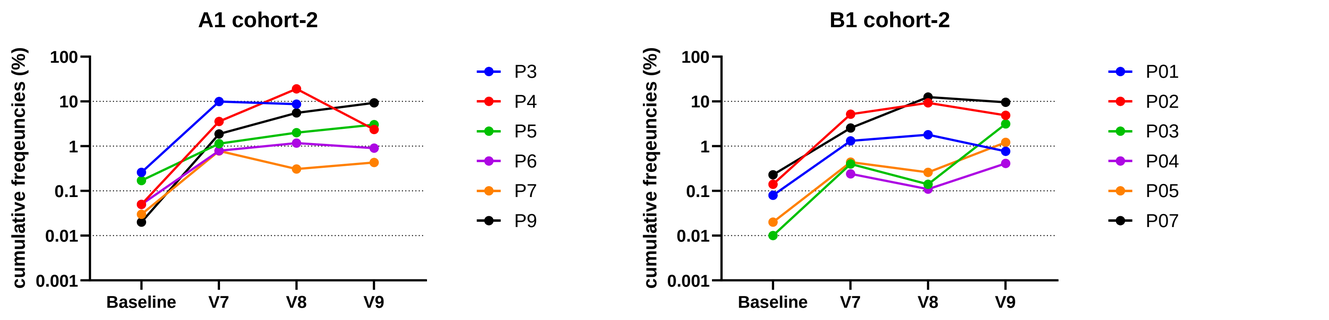
Patient #	P3	P4	P5	P6	P7	P9
Selected clonotypes	39	33	20	16	5	46



Patient #	P01	P02	P03	P04	P05	P07
Selected clonotypes	15	32	5	5	7	28
Clinical response*	PR	PR	PD	SD	PR	PR
Best change in target lesion (%)	-60	-85	-25	-15	-80	-70

*PR: partial response - SD: stable disease - PD: progressive disease

- **Cumulative frequencies of selected clonotypes in each patients:** the individual frequencies of each selected clonotype were added together for each timepoints



Conclusion

- The data generated by qTCRseq are comparable between the two cohorts and the different timepoints
- The analysis strategy revealed polyclonal amplifications of unknown specificity in the T CD8+ population during treatment with anti-tumour vaccines and immunomodulation
- The response is dynamic: the identity and number of selected clones are variable between patients and timepoints
- The cumulative frequencies of selected clonotypes range between 0.1% and 10% of total T CD8+ population and the expansion is still observed 10 weeks post-treatment
- Similar polyclonal amplifications were observed after treatment in stage IV patients treated with anti-PD1+PDC*lung01 in cohort B1, and in stage IIa to IIIa patients treated only with PDC*lung01 in the adjuvant setting in cohort A1.

Protocol Nb: PDC-LUNG-101, Status: Recruitment completed, Clinical Trial Identification: NCT03970746; This study is sponsored by PDC*line Pharma SAS. Contact info: j.plumas@pdc-line-pharma.com