Exploratory analysis of T cell repertoire dynamics upon systemic treatment with the oncolytic virus pelareorep in combination with pembrolizumab and chemotherapy in patients with advanced pancreatic adenocarcinoma (Abstract #2272)

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Abstract

Background: Pelareorep is an immuno-oncolytic virus that induces an inflamed tumor phenotype in metastatic adenocarcinoma of the pancreas (MAP). Systemically delivered pelareorep in combination with chemotherapy achieves 1 & 2 year-survival rates of 46% & 24% in MAP patients (pts), respectively[1]. Analysis of tumor tissue from pts treated with pelareorep, chemotherapy and anti-PD-L1 have shown reovirus RNA and protein replication, T cell infiltration, and upregulation of PD-L1, highlighting that effective T cell recognition of tumor antigens may be critical to success for this combination therapy[2]. Thus, we hypothesized that pelareorep in combination with chemotherapy and pembrolizumab in pts with MAP would alter the peripheral T cell repertoire, creating new T cell clones via the release of novel neoantigens in addition to expanding existing T cell clones.

Methods: A phase 1b study enrolled 11 MAP pts who progressed after first-line treatment. Pts received pelareorep (4.5e10 $TCID_{50}$ IV, D1 & D2), plus pembrolizumab (2 mg/kg IV, D8) plus either 1) 5-FU (LV 200 mg/m^2, 5-FU 200 mg/m^2 IV bolus, 5-FU 1200mg/m^2 continuous IV infusion D1) or 2) gemcitabine (1000 mg/m^2 IV, D1), or 3) irinotecan (125 mg/m^2 IV, D1) q3w, until disease progression/unacceptable toxicity. DNA from peripheral blood mononuclear cells from nine patients at cycle 1 day 1 (C1D1) & C2D1 (approx. 3 weeks later) was analyzed using the immunoSEQ® Assay (Adaptive Biotechnologies, Seattle) sequencing the T cell receptor beta chain region to interrogate changes in the T cell repertoire.

Results: The median Morisita index between C2D1 and C1D1 was 0.83 with three samples below 0.6, indicative of significant peripheral repertoire turnover. The median number of expanded clones equated to 45.7 per 100,000 cumulative templates; normal variation over 4 weeks is ~ 5-10 expanded clones. Strikingly, most (median: 86%) peripheral clonal expansion occurred in clones below the limit of detection at C1D1. Cox regression analysis revealed that high peripheral clonality correlates with progression free survival at C1D1 (HR = 0.053, p = 0.01). Moreover, high clonality correlates with overall survival at both C1D1 (HR = 0.124, p = 0.013) and C2D1 (HR = 0.079, p = 0.010).

Conclusions: High levels of peripheral T cell repertoire turnover occur between C1D1 and C2D1. Repertoire turnover is accompanied by significant clonal expansion, mostly by expansion of new clones (i.e. undetected in C1D1). Higher peripheral clonality is associated with better progression free survival at C1D1, and overall survival at C1D1 and C2D1. This research highlights the potential utility of T cell clonality as a predictive and prognostic biomarker to pelareorep therapy and warrants further clinical investigation.

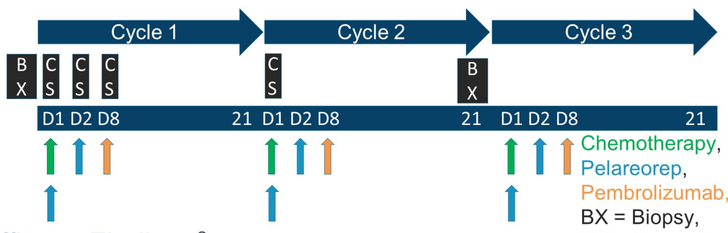
Background

Study Design and Schedule[2]

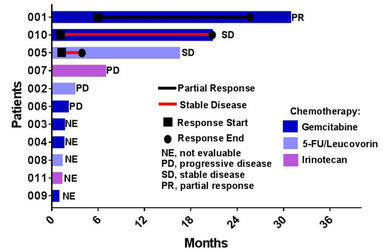
2L Relapsed Metastatic Adenocarcinoma of the Pancreas (MAP) n = 11

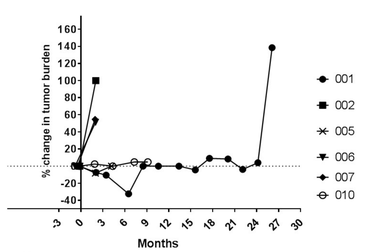
→ 21 day cycle:
- pelareorep 4.5x^{10} $TCID_{50}$ IV
- Pembrolizumab 2 mg/kg IV
- Gemcitabine or 5-flurouracil or irinotecan

→ Primary endpoint: •Dose-limiting toxicities Secondary Endpoints: ORR, PFS, OS, biomarkers



Efficacy Findings[2]





- Disease control was achieved in 50% of the 6 efficacy-evaluable pts.
- Of 6 efficacy evaluable pts, one achieved partial response (PR) 197 days after the start of therapy that has lasted 17.4 months.
- Two additional pts achieved stable disease (SD) 57-59 days on therapy, lasting 277 and 126 days, respectively.
- Nine pts have died secondary to progressive to disease (PD).

Background cont.

Safety Findings[2]

Treatment was safe and tolerable in all patients without an increase in Grade 4 toxicity (n = 11).

Preferred Term	Any grade	Grade 1/2	Grade 3	Grade 4
Any event, n (%)	10 (90.1)	10 (90.1)	1 (9.1)	1 (9.1)
Fever	9 (81.8)	8 (72.7)	1 (9.1)	0
Chills	6 (54.5)	5 (45.5)	1 (9.1)	0
Fatigue	3 (27.3)	3 (27.3)	0	0
Headaches	3 (27.3)	3 (27.3)	0	0
Anemia	2 (18.2)	2 (18.2)	0	0
Emesis	2 (18.2)	2 (18.2)	0	0
Flu-like Symptoms	2 (18.2)	2 (18.2)	0	0
Hypotension	2 (18.2)	2 (18.2)	0	0
Nausea	2 (18.2)	2 (18.2)	0	0
Neutropenia	2 (18.2)	2 (18.2)	0	1 (9.1)
Leukopenia	1 (9.1)	0	0	1 (9.1)

Treatment-related adverse events occurring at any grade in ≥ 10% of patients or grade ≥ 3 in any patient

Study Hypothesis

We hypothesized that pelareorep in combination with chemotherapy and pembrolizumab in pts with MAP would alter the peripheral T cell repertoire, specifically we asked:
- If pelareorep (oncolytic virus) treatment creates novel T cell clones via release of neoantigens, or
- If pelareorep treatment expands existing T cell clones.

Methods

Immuno sequencing of the CDR3 regions of human TCRβ chains was performed using the ImmunoSEQ® Assay developed by Adaptive Biotechnologies, Seattle, WA. DNA for this assay was isolated from PBMCs collected at cycle 1 day 1 (C1D1), C1D8, and C2D1. Diversity is calculated as Shannon's Entropy by:

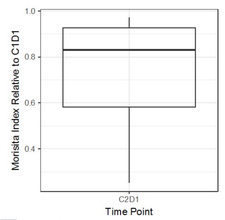
$$Diversity = H = -\sum_{i=1}^{N} p_i \log_2(p_i)$$

Where p_i is the proportional abundance of clone i, and N is the total number of unique receptor gene rearrangements. Clonality is defined as 1 − Pielou's evenness metric and is calculated by 1 − H/ln(N). Where indicated, Simpson Clonality is defined as $\sqrt{\sum p_i^2}$ where p_i is the frequency of each rearrangement in the repertoire.

Results

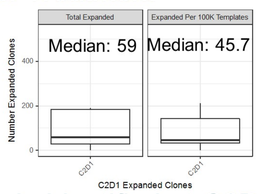
Low Morisita Indices Between C1D1 and C2D1 Suggests High Repertoire Turnover

- Morisita Index takes into account both repertoire overlap and clonal frequencies between the two samples. A perfectly identical repertoire is 1, and two completely disparate samples would be 0.
- Normal variation over a month is ~0.9– 0.95.
- The median Morisita between C2D1 and C1D1 is 0.83 with 3 samples below 0.6. This suggests significant peripheral repertoire turnover.



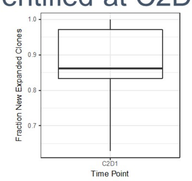
Significant Peripheral Clonal Expansion Over Treatment

- Peripherally expanded clones were determined between C1D1 and C2D1.
- Normal variation over 4 weeks is ~ 5-10 expanded clones.
- Median values are greater than 40 in both cases. Only 1 sample had less than 18 expanded clones.



Most Peripherally Expanded Clones are Newly Identified at C2D1

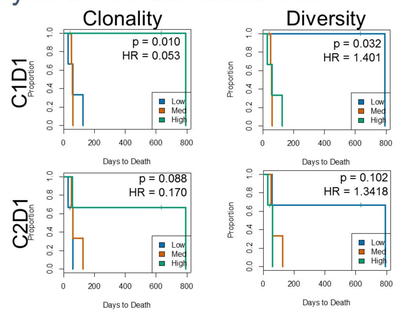
- Peripherally expanded clones can be either expansion of existing clones or newly identified clones (i.e. undetected in the first time point).
- Most peripheral clonal expansion is observed from new clones (Median: 86%).



Results cont.

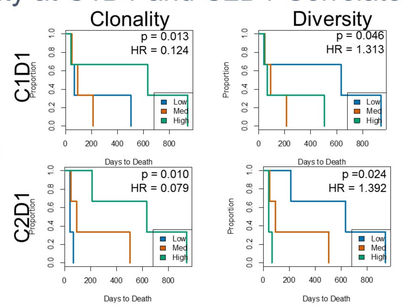
Peripheral Clonality and Diversity at C1D1 Correlate with Progression Free Survival

- Variables were treated as continuous variables for cox regression.
  - Clonality was scaled to a unit of 0.1
  - Diversity was scaled to a unit of 100
- Clonality and diversity are correlated with progression free survival and show a stronger p-value at C1D1.
- Higher peripheral clonality and lower diversity are associated with longer progression free survival.



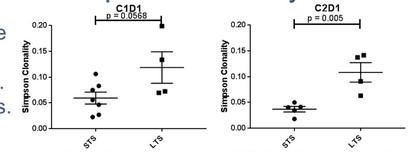
Peripheral Clonality and Diversity at C1D1 and C2D1 Correlate with Survival Time

- Variables were treated as continuous variables for cox regression.
  - Clonality was scaled to a unit of 0.1.
  - Diversity was scaled to a unit of 100.
- Clonality and diversity are correlated with overall survival and show a stronger p-value at C2D1.
- Higher peripheral clonality and lower diversity are associated with better outcome.

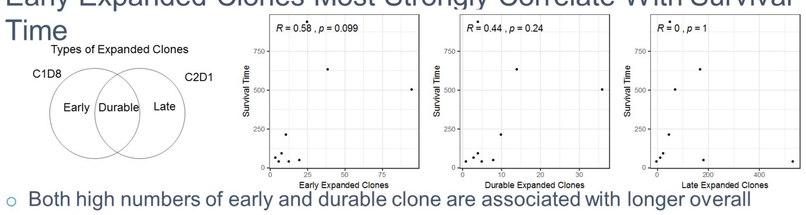


Long Term Survivors Have Greater Peripheral Clonality at C2D1

- Responders and long term survivors have greater peripheral Clonality at C2D1.
  - Long term survivors (LTS): > 6 months.
  - Short term survivors (STS): < 6 months.



Early Expanded Clones Most Strongly Correlate With Survival Time



- Both high numbers of early and durable clone are associated with longer overall survival times.
- The strongest correlation is seen with the number of early expanded clones.
- Early vs. late clonal expansion may be influenced by the type of response pelareorep is eliciting.

Conclusion

- Higher peripheral clonality and lower diversity are associated with better overall survival.
- High levels of peripheral repertoire turnover occur between C1D1 and C2D1.
- Repertoire turnover is accompanied by significant clonal expansion, mostly by increases in "new" clones (clones that were undetected in C1D1).
- The number of early expanded clones (prior to pembrolizumab) is associated with longer overall survival. There is no correlation with either durable or late expanded clones and clinical outcome.
- A study by Hopkins *et al*. has also shown that peripheral T cell repertoire associates with survival in MAP pts treated with nivolumab and a pancreatic cancer vaccine[3].

References

1. Mahalingam, D., et al., *A Phase II Study of Pelareorep (REOLYSIN((R))) in Combination with Gemcitabine for Patients with Advanced Pancreatic Adenocarcinoma*. Cancers (Basel), 2018. **10**(6).
2. Mahalingam, D., et al., *A study of REOLYSIN in combination with pembrolizumab and chemotherapy in patients (pts) with relapsed metastatic adenocarcinoma of the pancreas (MAP)*. J Clin Oncol 35, (suppl; abstr e15753), 2017.
3. Hopkins, A.C., et al., *T cell receptor repertoire features associated with survival in immunotherapy-treated pancreatic ductal adenocarcinoma*. JCI Insight, 2018. **3**(13).

See poster online: