Comparative Assessment of Immune Biomarkers from Phase 1 First-in-Human Trial Treating Advanced Cancer Patients with DK2[10] (EGFR) to *ex vivo* Diakine™ Response Assay

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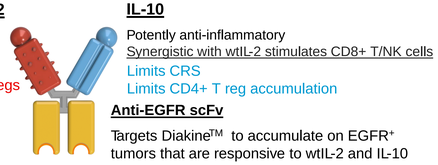
Abstract # 43

Trial Registration:
(NCT05704985)

BACKGROUND

- Diakine™ DK2[10] (EGFR) couples wild-type IL-2 (wtIL-2) to an IL-10 high affinity variant via a scaffold (scFV) that binds Epidermal Growth Factor Receptor (EGFR) (Fig 1)
- Coupling wtIL-2 with IL-10 removes the toxicity associated with wtIL-2 and improves the potency of the molecule, while targeting the molecule to the tumor cell surface within the tumor microenvironment (TME) improves effectiveness
- Utilizing an *ex vivo* response assay, biomarkers demonstrating safety and potency were examined. This assay corroborates patient response in the phase 1 (NCT05704985) dose escalation study

Figure 1 – Structure of DK2[10] (EGFR): Cytokines and Targeting System Orient to Opposite Sides of the Molecule



METHODS

- Subjects with relapsed/refractory solid tumors known to express EGFR were enrolled (Table 2)
- Dose escalation through 16 mg three times weekly (TIW) self-administered subcutaneous injection (Fig 2)
- Evaluation of biomarkers for safety and potency
- Fold changes evaluated at baseline to highest change or day 22
- Data presentation is restricted to the initial 31 patients, with a data cut-off of September 20, 2024

Figure 2 – Study Design

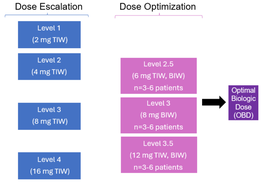


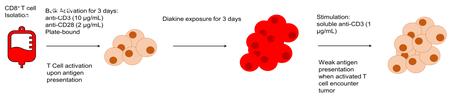
Table 1 - Treatment Schedule

DK2[10] (EGFR) administered subcutaneously (SC)	D1, 3, 5 (TIW) or D1, 4 (BIW) every week (cycle = 3 weeks)
PK/PD sampling	Cycle 1: D1-5, D8; Cycle 2: D1-2; then concurrent with response evaluation
Response Evaluation	CT/MRI every 9 weeks

Table 2 – Demographics and Baseline Characteristics

Age (years)	Median 65; Range 45-79
Sex	Male n=16 (52%); Female n=15 (48%)
Diagnosis	RCC n=11 (36%) CRC n=9 (29%) NSCLC n=6 (19%) PDAC n=5 (16%)
Race	White n=17 (55%) African American n=4 (13%) Asian n=2 (6%) Native Hawaiian n=1 (3%) Unreported n=7 (23%)
Prior Therapy	Chemotherapy: 31/31 (100%) CPI: 15/31 (48%)
Mutational Burden	14 (100%) of CRC/PDAC-MS Stable

Figure 3 – IFNγ Predictive Response Assay



- CD8+ T cells are model antigen bulk activated for 3 days, exposed to DK2[10] (EGFR) for 3 days, then triggered with anti-CD3 to induce Interferon-γ (IFNγ) secretion Secreted (Fig 3)
- Secreted IFNγ levels denote responders (high) vs. non-responders (low)
- The genetic differences between the groups are under investigation

HYPOTHESES:

- Cytokine profile: IFNγ will be induced without significant upregulation of other inflammatory cytokines that would result in VLS or CRS. IP-10, wtIL-2Rα, IL-18, and IL-18 binding protein will also increase
- wtIL-2 will induce IL-5 and result in eosinophilia
- Peripheral T cell and NK cell proliferation will be induced without upregulation of Tregs
- Immune system reprogramming will enable new T cell and NK cell anti-tumor response and can be measured by an increased in new T cell clones

RESULTS

Figure 4 – Results of Response Assay: CD8+ T Cell Dose Response to DK2[10] (EGFR)

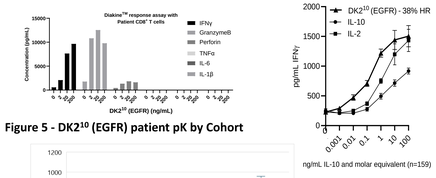
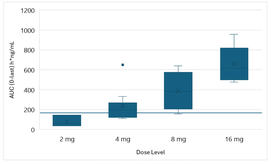


Figure 5 - DK2[10] (EGFR) patient pK by Cohort



- DK2[10] (EGFR) dose-dependently (0-100 ng/ml) induced anti-tumor cytotoxic molecules (IFNγ, Granzyme B [GzmB], and perforin) without marked increases in TNFα (Fig 4)
- The magnitude of IFNγ induced was higher than benchmarks reported for wtIL-2, without inducing TNFα
- Combining IL-10 with wtIL-2 uncouples IFNγ induction from IL-6, IL-1β, and TNFα

Evidence of wtIL-2 signaling at all dose levels

- Pre-clinical studies defined target AUC > 150 h*ng/mL for clinical activity
- AUC exposure achieved in 2 mg dose cohort of ~145 h*ng/mL with confirmed 6-month stable disease (Fig 5)
- Exposures are ~4X PEG-IL-10 and 2X high dose wtIL-2 AUC
- On treatment patient plasma showed IFNγ increased 20-fold from cohort 1 to 3 but plateaued at the 8 mg dose (Fig 6)

Figure 6 – Cytokine Profile Across Cohorts

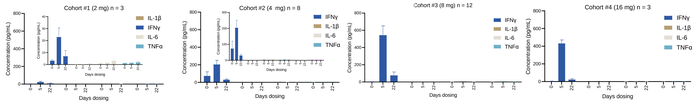
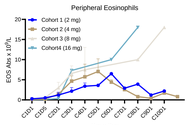
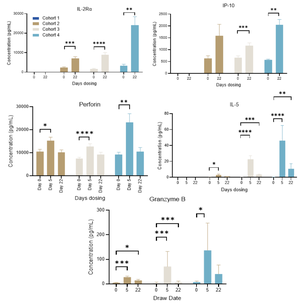


Figure 7 – Eosinophils by Cohort



- Asymptomatic eosinophilia was observed but no clinical intervention required (Fig 7)
- Treatment with DK2[10] (EGFR) leads to the induction of wtIL-2 biomarkers IFNγ, wtIL-2Rα, IP-10 and IL-5 in all cohorts (Fig 8)
- GzmB and perforin increased dramatically in plasma, but not CRS-associated cytokines TNFα, IL-1β, and IL-6

Figure 8 – Induction of wtIL-2 Biomarkers in All Cohorts



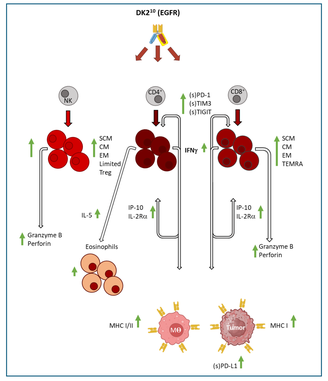


Figure 9 – Induction of Checkpoint Inhibitors in Plasma of DK2[10] (EGFR) Treated Patients

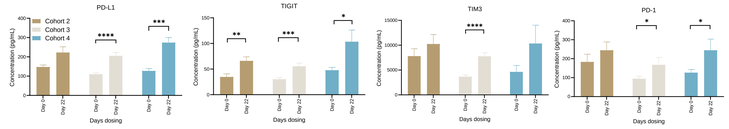
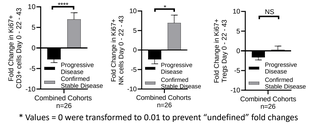
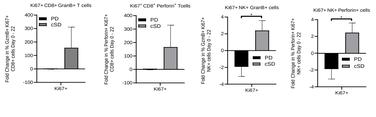


Figure 10 – Increase in CD3+ and NK Cell Proliferation Without Increase in Tregs



* Values = 0 were transformed to 0.01 to prevent "undefined" fold changes

Figure 11 – Granzyme B and Perforin Upregulation in both CD8+ T cells and NK cells (D0 vs D22)



- DK2[10] (EGFR) induces expansion of CD3+ T and NK cells, but not Tregs in patients exhibiting stable disease (Fig 10)
- wtIL-2 and IL-10 are known to control Granzyme and perforin, which are increased in patient T and NK cells along with Ki-67 (an indication of proliferation) (Fig 11)
- TCRβ sequencing was conducted in a subset of patients showing immune activation correlated with clonal expansion and enhanced repertoire diversity starting at Day 5 (Fig 12)
- Changes in peripheral repertoire are correlative with precision patient selection assay results

Figure 12 – Clonal Expansion and Enhanced Repertoire Diversity in Immune "Responders"

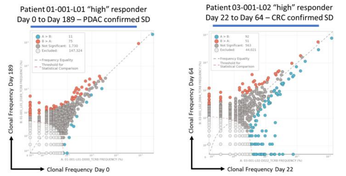


Table 4 – Hypotheses Proven

Hypothesis:	Clinical Proof:
Achieve therapeutic exposure level of 200 (h*ng/mL)	Minimal therapeutic exposure achieved in dose levels 2-4 (4-8 mg TIW)
Evidence of wtIL-2 signaling	Eosinophilia without clinical sequelae
Ameliorate cytokine release syndrome (CRS)	Low frequency, low grade CRS reported Low frequency, low grade hypotension reported No pro-inflammatory cytokines associated with CRS (IL-1β, IL-6, TNFα)
Signaling of immune response	Induction of peripheral CD3+ T and NK cell proliferation/accumulation but not Tregs and new T cell clones expanding

CONCLUSIONS

- The immune response biomarker profile in patients in the Response Assay to DK2[10] (EGFR) and the on-treatment assessment demonstrated that coupling wtIL-2 with IL-10 and targeting within the TME results in potent immune activation without inducing cytokines that drive significant systemic toxicity or statistically significant increase in Tregs
- These data confirm the potent, balanced, and targeted hypothesized mechanism of action of DK2[10] (EGFR) in patients
- This proof of mechanism supports further clinical evaluation of DK2[10] (EGFR) in RCC and NSCLC and validates the Diakine™ platform.
- Further exploration of DK2[10] (EGFR) to optimize monotherapy dose selection is ongoing before proceeding to evaluate clinical activity in expansion cohorts and relevant combinations

Acknowledgements:

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