(765) DNase I Targeting of Neutrophil Extracellular Traps Improves CTLA-4 Immune Checkpoint blockade in Models of MSS/MMRp CRC

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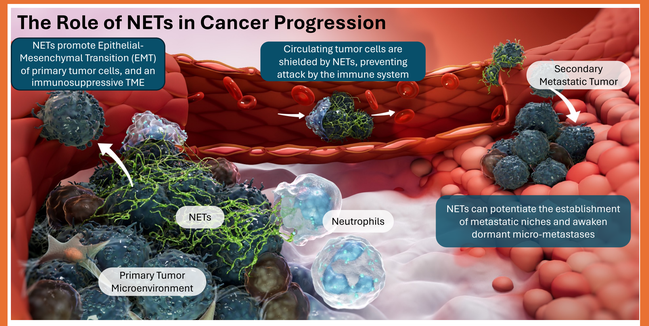
The Role of NETs in Cancer Progression



BACKGROUND

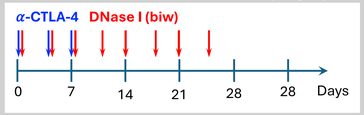
Neutrophil Extracellular Traps (NETs), are a form of cell-free DNA (cfDNA) released by activated intratumoral neutrophils. In tumors:

- Excessive NET formation is linked to increased tumor growth, metastasis, the awakening of dormant metastases.
- NETs can shield tumor cells, induce expression of immunosuppressive cytokines (e.g., TGFβ), cause CD8+ T cell dysfunction, and promote the activity of regulatory T cells (Tregs).

Several human cancers, and gastrointestinal cancers in particular (e.g., colorectal, CRC) have high levels of neutrophil infiltration and NETs, which contribute to an immunosuppressive, protumor microenvironment (TME), leading to poor response to therapies. In CRC, where the majority of patients (~85%) are microsatellite stable (MSS), mismatch repair proficient (MMRp), immune checkpoint inhibitors (ICIs) have shown very little clinical activity. There are several reasons for this, but a major issue is an immunosuppressive TME, to which NETs contribute significantly.

MATERIALS & METHODS

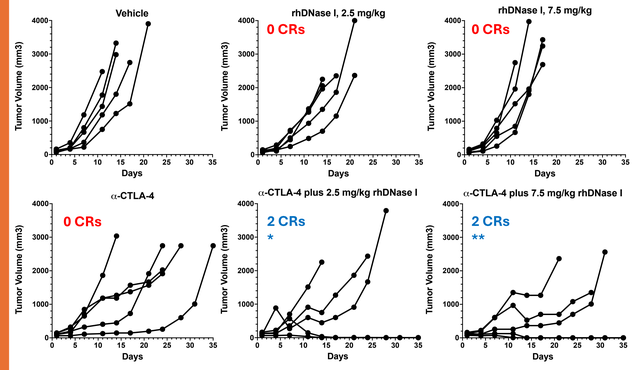
1. Female BALB/c mice 8 to 12 weeks at start.
2. 3 x 10^5 CT26 or 1 x 10^6 Colon26 tumor cells subcutaneously (sc).
3. Randomized when tumors reached a mean tumor volume (MTV) of 80 - 120 mm^3 and treatment started (day 0).



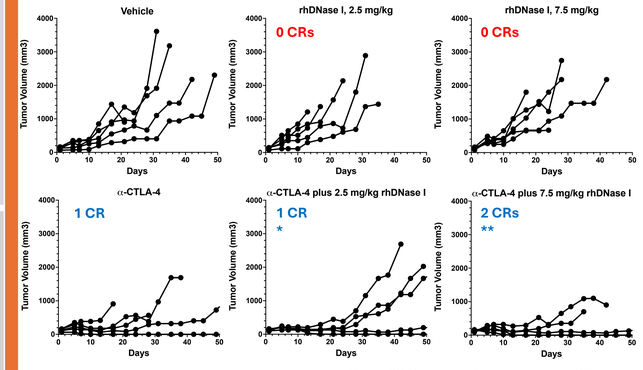
4. Anti-CTLA-4 (clone 9H10; BioXcell).
5. For rechallenge studies, mice which had experienced complete responses (CRs) were monitored for an additional 28 days and then rechallenged with CT26 or Colon26 tumor cells.

FIGURE 3. DNase I plus α-CTLA-4 combination therapy results in tumor growth inhibition, several CRs and enhanced survival in mice bearing CT26 or Colon26 microsatellite stable, mismatch repair proficient (MSS/MMRp) colorectal carcinoma (CRC) tumors.

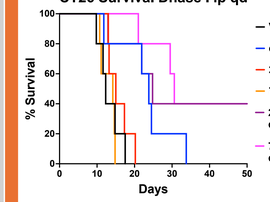
CT26 MSS/MMRp CRC



Colon26 MSS/MMRp CRC



CT26 Survival Dnase I ip qd



Colon26 Survival DNase I ip qd x 20

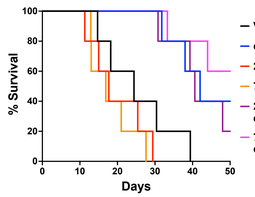
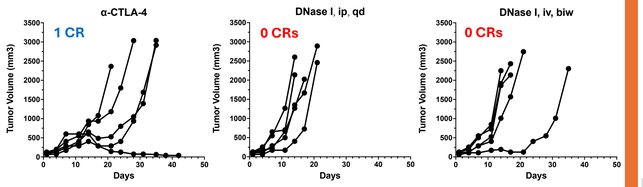
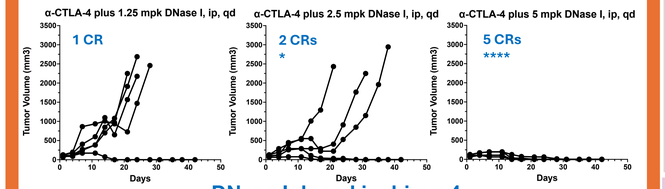


FIGURE 4. Dose response of DNase I combined with α-CTLA-4, with an evaluation of both route and frequency of administration, in the CT26 model. DNase I plus α-CTLA-4 combination therapy resulted in complete responses (CRs) in mice bearing CT26 tumors.

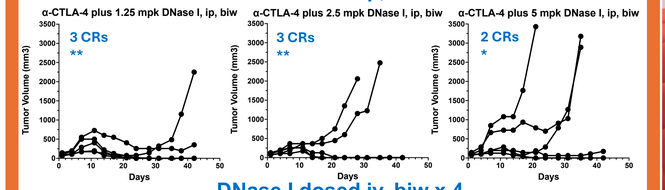
α-CTLA-4, DNase I Controls



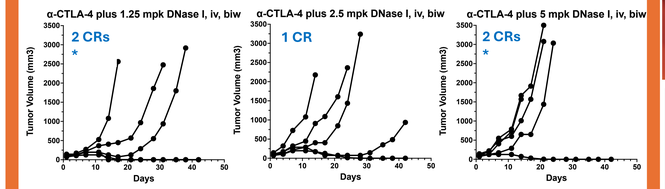
DNase I dosed ip, qd x 20



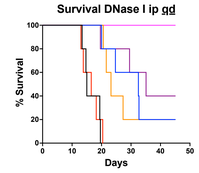
DNase I dosed ip, biw x 4



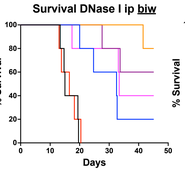
DNase I dosed iv, biw x 4



Survival DNase I ip qd



Survival DNase I ip biw



Survival DNase I iv biw

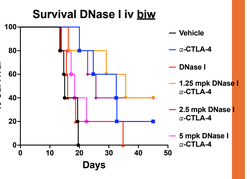
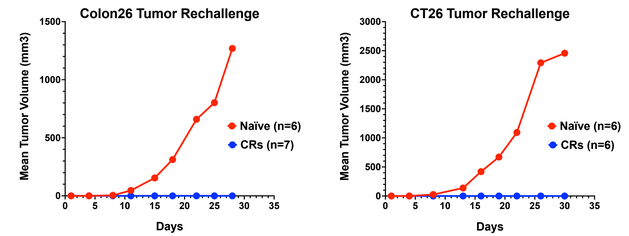


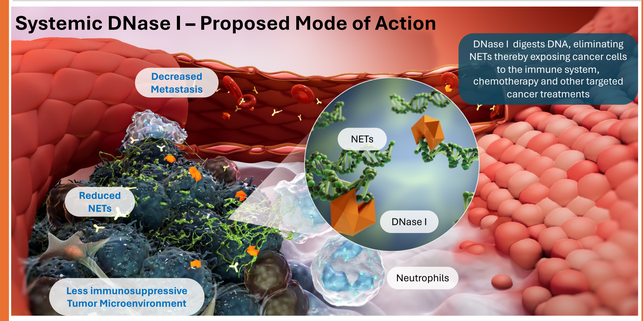
Figure 5. Rechallenge of Colon26 and CT26 complete responders results in no (0 mm3) tumor take or growth



SUMMARY.

1. DNase I plus α-CTLA-4 combination therapy results in tumor growth inhibition, complete responses (CRs) and enhanced survival in mice bearing CT26 or Colon26 microsatellite stable, mismatch repair proficient (MSS/MMRp) (CRC) tumors.
2. When CT26 or Colon26 tumor-bearing mice experiencing complete tumor rejection are rechallenged with the same tumor cells 28 days later, the tumor cells are rejected and fail to take.

Systemic DNase I – Proposed Mode of Action



MOVING FORWARD.

1. The data suggests that systemic DNase I combined with α-CTLA-4 antibody promotes antitumor immunity and generates immunological memory against microsatellite stable, mismatch repair proficient (MSS/MMRp) colorectal carcinoma (CRC) tumors.
2. Preliminary data suggests that DNase I impedes neutrophil tumor infiltration, promotes CD4 and CD8 T cell infiltration, and enhances intratumoral T cell activation.
3. Future efforts will focus on additional immune checkpoint inhibitor combinations (e.g., α-PD-1, etc.) and tumor-infiltrating lymphocyte (TIL) analysis employing digital spatial profiling