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PIN-2, A Novel Immunomodulatory Peptide

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Abstract

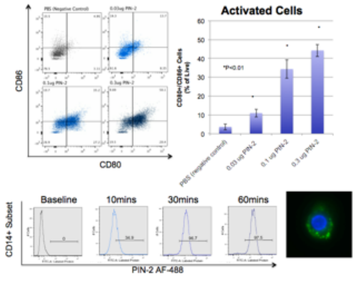
PIN-2 is a novel immunomodulatory agent derivatized from a transactivator protein. We have shown its activity to induce maturation of monocytes into dendritic cells and translational proof of principle using an aggressive metastatic murine mammary cancer model. The purpose of the present studies was to further elucidate the activity and mechanism of action of PIN-2 alone or in combination with other agents in the treatment of solid malignancy. Murine mammary carcinoma was established with syngeneic 4T1 cells orthotopically implanted in the mammary fat pad of female BALB/c mice and allowed to seed 7 or 10 days before starting treatment. PIN-2 was given as an intravenous bolus of 40-100 ng/mouse on alternating weeks. ***Experiment 1***: Combination therapy with PIN-2 and cyclophosphamide (CTX). Treatment began on d10, when tumor diameter was ~ 4-5mm. There were 4 treatment arms (n=10): Placebo control (PBS IV every 3 days), CTX control (CTX 80 mg/kg IP once/wk), combination sequence 1 (Seq1:PIN-2 40ng IV every 3d/CTX, alternating 10d cycles), and combination sequence 2 (Seq2: CTX/PIN-2, alternating 10d cycles). Treatment was stopped on day 50. Tumor size was measured 3 times/wk. End points were tumor volume and overall survival. ***Experiment 2***: PIN-2 (100ng IV 3x/wk) and α-CTLA-4 (200µg IP 3x/wk) were administered to tumor bearing mice. There were 4 treatment arms (n=10): control, α-CTLA-4 (week 2 only), PIN-2 (weeks 1 and 3) and PIN-2 (weeks 1 and 3) followed by α -CTLA-4 (week 2). Animals were killed on day 29/30. End points were tumor progression and number of spontaneous lung surface nodules. Pharmacodynamic end points were primary tumor staining for PD-L1 and CD8. FFPE primary tumors from control and PIN-2 treated mice were stained using IHC for PD-L1 and CD8. Determination of PD-L1 reduction is based on *in vivo* tumor measurements together with PD-L1 staining intensity. **Experiment 1**: Both PIN-2 and CTX impacted tumor growth and increased overall survival followed the order Seq1 (PIN-2/CTX)>Seq2 (CTX/PIN-2)> CTX. All mice treated with PIN-2 had reduced tumor burden vs CTX alone or control. **Experiment 2**: Both PIN-2 and combination therapy, but not α -CTLA-4 alone, were effective in reducing tumor burden vs control. Additionally, the PIN-2/α -CTLA-4 combination therapy resulted in fewer lung surface nodules than α -CTLA-4 alone or control. IHC showed that PD-L1 expression is reduced in primary tumors from animals receiving PIN-2 vs controls. Tumor edge contained PD-L1+ staining was largely absent in PIN-2 treated animals vs control. Rarely, CD8+ cells were seen in PBS control tumors whereas significant CD8+ staining was observed around tumor edges in PIN-2 treated mice. CTX activity is improved when given in combination with PIN-2. CTX is most effective when administered after PIN-2. The MOA of PIN-2 suggests a priming effect linking innate and adaptive immunity. PIN-2 and α -CTLA-4 were both effective in reducing the average lung surface nodule count. However, when PIN-2 is combined with α -CTLA-4, the combination is more effective than either compound alone. This suggests a priming effect of innate immunity that enhances α -CTLA-4 activity. PD-L1 expression is reduced in PIN-2 treated primary 4T1 tumor and promotes the influx of tumor infiltrating CD8+ CTL. Tumor-infiltrating CD8+ CTLs localize near the tumor edge in PIN-2 treated mice, whereas these CTLs are largely absent in tumor PBS controls. Since PD-L1 is a marker associated with disease progression, malignancy, and poor prognosis, the inverse correlation of tumor PD-L1 and CD8+ CTL can be explained by the antitumor CTL response seen with PIN-2 treatment. In conclusion, PIN-2, a novel immunomodulatory peptide, demonstrated immune priming activity capable of linking the innate and adaptive immune systems thereby enhancing and promoting anti-tumor activity by triggering monocyte derived dendritic cells to stimulate CD8+ CTL responses.

Objectives

1. **To demonstrate anti-tumor and anti-metastatic activity of PIN-2 in established-poorly immunogenic metastatic 4T1 murine mammary carcinoma model either alone or in combination with other anti-cancer agents.**
2. **To demonstrate an increase in CD8+ tumor infiltrating lymphocytes (TIL's) in response to PIN-2 treatment.**
3. **To explore the efficacy of PIN-2 in the context of PD-L1 /B7-H1 induced immunosupression.**

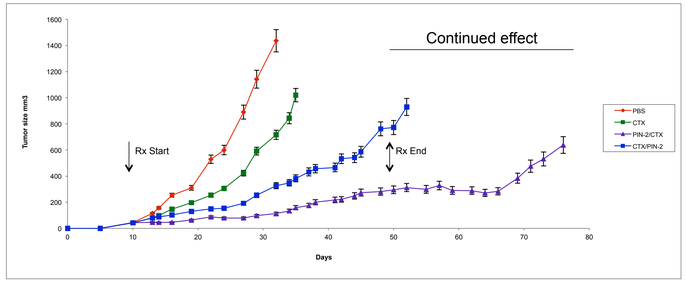
Background

- ***PINS* are Transactivator protein derivatives.**
- **PIN-2 is a cell penetrating peptide that triggers stimulation and maturation of CD14+ human monocytes to activated antigen presenting cells. (Shown right →)**
- ***PINS* drive the DC-APC innate immunity complex to stimulate cytotoxic T lymphocytes linking innate (CD80/CD86) and adaptive immunity (CD8).**
- ***PINS* act upstream of immune checkpoints through the innate immunity complex.**



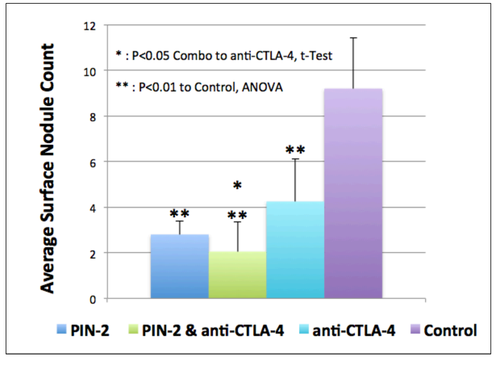
Methods & Results

"*Immunopriming*" with PIN-2 and Alternating Cyclophosphamide Rx Impacts Established Solid Tumor Progression.



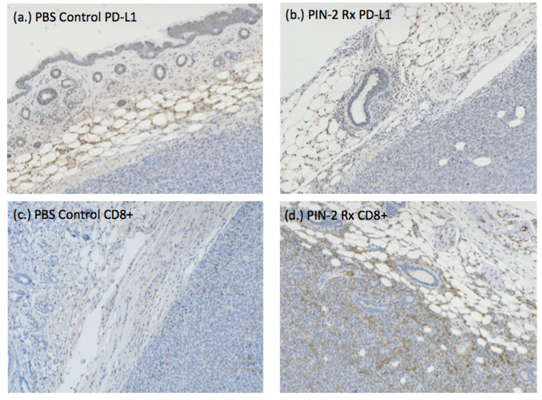
Above: Experiment 1 depicts 4T1 tumor growth in mice following treatment with repeat dosing in alternating 10-day treatment cycles with PIN-2 and Cyclophosphamide. Treatment begins on day 10 when primary tumor was established at 4-5 mm^3 and discontinued on Day 50: The PIN-2/CTX combination demonstrated the most meaningful impact on overall survival and continued impact on tumor growth.

Combination PIN-2 + α-CTLA-4 Rx Reduce Distant Site Metastasis.



Above: Experiment 2 depicts Average number of pulmonary surface nodules from mice treated with PIN-2, Combination PIN-2 & α-CTLA-4, α-CTLA-4, or PBS control. The addition of bolus PIN-2 to α-CTLA-4 resulted in fewer numbers of surface nodules on Day 30 compared to α-CTLA-4 standalone therapy. → *enhanced responsiveness.*

PIN-2 Overrides PD-L1/B7-H1-Mediated Adaptive Immune Resistance in a Solid Tumor Microenvironment.



← Experiment 2 depicts BALB/C mice were implanted with 4T1 cells in the mammary fatpad and treated with PIN-2 or PBS. Immunohistochemical staining of FFPE primary tumor tissue taken from day 29 and stained for (a.) PBS-control PD-L1/B7-H1. (b.) PIN-2 Rx PD-L1/B7-H1. (c.) PBS-control CD8+. (d.) PIN-2 Rx CD8+. Tumor-infiltrating CD8+ CTLs appear to localize near the tumor edge in PIN-2 treated mice, where as these CTLs are largely absent in tumor edges of PBS control. Since PD-L1 is a marker associated with disease progression, malignancy, and poor prognosis (*"molecular shield"*), the inverse correlation of tumor PD-L1 and CD8+ CTL can be explained based on the antitumor CTL response observed with PIN-2 treatment.

Conclusions

- **PIN-2 is a novel *immunomodulatory* peptide with a rapid onset of action that stimulates innate immunity by activating APCs *de novo*.**
- **Activation of the innate immunity complex by PIN-2 stimulates an enhanced endogenous adaptive T-cell response as evidenced by an increase in CD8+ CTL → "*immunopriming*".**
- **PIN-2 breaks T-cell anergy evidenced by the inverse correlation of PD-L1 and CD8+ PIN treated 4T1 primary tumors → overrides the immunosupressive PD-L1/B7-H1 "*molecular shield*".**
- **PIN-2 immunomodulatory activity downshifts primary tumor growth, significantly reduces metastases, and increases overall survival → *primes and focuses* the innate immune system to drive an adaptive endogenous T-cell response.**
- **PIN-2 synergizes with other counter suppressive anticancer therapeutics (CTX, α-CTLA-4) predicated upon physiological mechanisms of the innate and adaptive immune responses → facilitates *enhanced responsiveness.***
- **PIN-2 immunotherapy rejuvenates the cancer-suppressed immune system.**
- **These data support clinical investigation of PIN-2 as an immunopriming/immunomodulatory agent with other treatment modalities to treat solid human cancers.**

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