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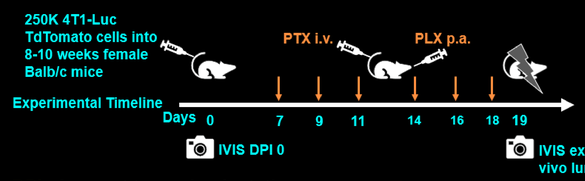
Background and Purpose

Lung cancer is the leading cause of cancer death among both men and women in the US and worldwide.[1] In spite of recent advances in the treatment of lung cancer including targeted and antibody therapies, 5 year overall survival of lung cancer patients continues to be very low at ca. 19%.[2] The purpose of this work was to evaluate the efficacy of a locally administered small molecule colony stimulating factor 1 receptor inhibitor (CSF-1Ri), PLX3397 (PLX), alone or in combination with cytoreductive therapy (paclitaxel, PTX) in reducing the tumor burden of an *in vivo* model of secondary lung cancer. CSF-1Ri have been shown to inhibit M2-like (tumorigenic) tumor-associated macrophages (TAMs or M2 Mϕ), and alone or in combination with chemotherapies, to reduce tumor burden in pre-clinical and clinical studies of various types of primary and secondary cancers.[3,4] Local administration of immunotherapy to the lungs may enhance lung biodistribution of such therapies, and reduce potential unwanted off-target toxicity. In addition, combination of such therapy with low dose standard of care chemotherapy may offer improved anti-tumor effects.

Methods

Pre-clinical Model of Secondary Lung Cancer



DPI: day post tumor implantation
IVIS: in vivo imaging system
Terminal day: day 19
Vehicle: 1% DMSO, 5%Tween80 in PBS
Pulmonary administration (p.a.): intratracheal installation (i.t.)

- **Treatment:**
  - Control: vehicle i.t.
  - Immunotherapy: PLX i.t.
  - Chemotherapy: PTX i.v.
  - Combination: PLX i.t. + PTX i.v.

all @ 1 mg/kg

References

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2. Cancer.net, last updated: 01.2019. https://www.cancer.net/cancer-types/lung-cancer-non-small-cell/statistics.
3. Zhu Y, et al. Cancer Research. 2014;74:5057–5069. doi: 10.1158/0008-5472.CAN-13-3723.
4. Cannarile MA, et al. J Immunother Cancer. 2017;5(1):53. doi: 10.1186/s40425-017-0257-y.

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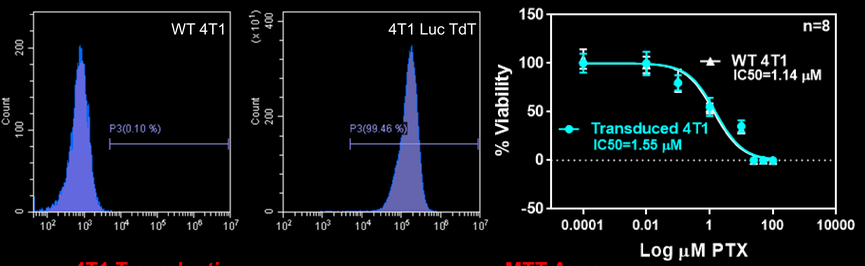
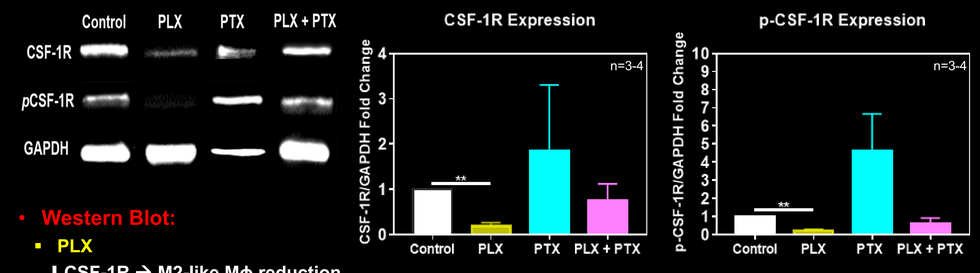
Results: Effect of Immunochemotherapy on Tumor Burden and Tumor Microenvironment (TME)

4T1 Transduced and no Changes in Response to PTX



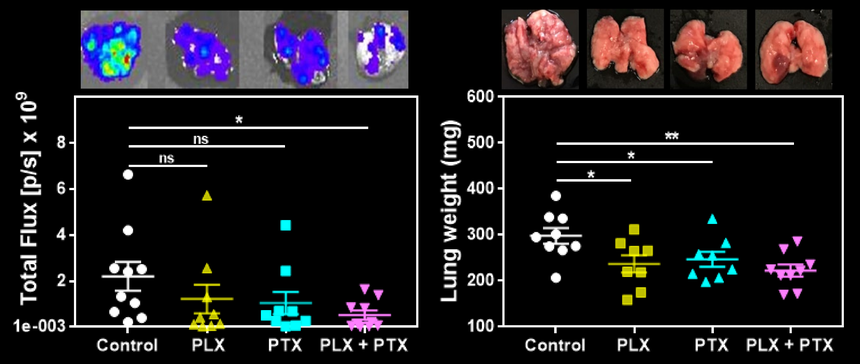
- **4T1 Transduction:**
  - Transduction w/ Lentivirus expresses luciferase (Luc), TdTomato (TdT), & puromycin resistant gene → >99% TdT
- **MTT Assay:**
  - PTX induced cell kill in WT 4T1 and 4T1 Luc TdT cells → transduced cells behave as WT

PLX p.a.: Reaches its Molecular Target in TME



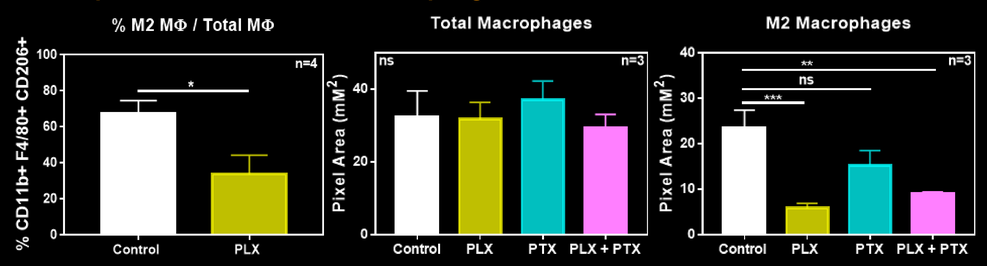
- **Western Blot:**
  - **PLX**
    ⬇ CSF-1R → M2-like Mϕ reduction
    ⬇ *p*CSF-1R → ⬇tumorigenic response
  - **PLX + PTX**
    PTX negatively affects TME → increases CSF-1R and *p*CSF → M2 Mϕ recruitment?

Combination Immunochemotherapy: Efficacious and Safe



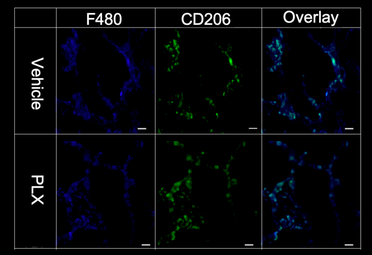
- **Tumor Burden (n=9-10):**
  - PLX or PTX → decreased burden (weight)
  - PLX + PTX → additive effect → further reduction in tumor burden
- **Toxicity:**
  - All treatment groups → no severe toxicity as per body weight, fur appearance, movement, staining around eye/nostrils, respiration, behavior.

PLX p.a.: Decreases M2-like Macrophages in TME



- **Immunofluorescence and Flow Cytometry:**
  - PLX → downregulated ($CD11b^+$) $F480^+CD206^+$ → ⬇M2 Mϕ
  - PLX + PTX → downregulated $F480^+CD206^+$ → ⬇M2 Mϕ
  - PTX → no effect on M2 Mϕ

  All therapeutics → no effect on total Mϕ



Conclusions

- **Local administration of immunotherapy to the lungs (PLX p.a.) supports chemotherapy (PTX i.v.) of breast to lung metastases:**
  - PLX + PTX reduced tumor burden (ex vivo IVIS & lung weight)
  - PLX reached its molecular target, Mϕ in TME
  - PLX reduced M2 Mϕ → shifted the balance towards anti-tumorigenic Mϕ phenotype

The da Rocha Group