Intratumoral injection of INT230-6 induces protective T cell immunity

Anja C. Bloom[1], Lewis H. Bender[2], Ian B. Walters[2], Masaki Terabe[1], Jay A. Berzofsky[1]

[1] Vaccine Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, USA; [2] Intensity Therapeutics, Westport, CT, USA

Abstract:

Standard care for many types of cancer involves systemic administration of cytotoxic agents. This may result in low drug concentration at tumor sites, which limits cell killing. More recently it has been shown that cytotoxic formulations designed for intratumoral delivery improve drug efficacy presumably by increasing drug concentration at the tumor site. Furthermore, it has been revealed that the mechanisms of anticancer agents extend beyond direct tumor cell lysis. One major aspect is that cell death often induces an immune response. Different types of cell death such as necrosis and autophagic cell death induced by cytotoxic agents trigger immune responses with varying degrees of inflammation and involving different types of immune cells. The ideal immune responses that may give maximum benefit to patients would be strong and long lasting anti-tumor T cell responses. In this study, a novel, tissue and cell diffusive cytotoxic formulation, INT230-6, was administered intratumorally over 5 sequential days into subcutaneous 300mm^3 murine Colon26 tumors. Treatment resulted in regression from baseline of 100% of the tumors and up to 80% complete response (CR). We then sought to analyze the T cell responses in the protection induced by INT230-6. Mice with CR were protected from re-challenge either by subcutaneous or intravenous re-inoculation of the Colon26. The protection was abrogated by CD4/CD8 double depletion prior to the re-challenge, indicating that immunological memory was induced. Colon26 tumors express the endogenous retroviral protein gp70 containing the AH-1 CTL epitope. AH-1-specific CD8 T cells were detected ex vivo in systemic organs such as spleens and peripheral blood of a subset of mice with CR, confirming induction of CD8 T cell specific responses to tumor cells upon INT230-6 treatment. Hence, INT230-6 given locally to treat tumors induces tumor specific protective T cell immunity.

Introduction:

Cytotoxic agents are frequently given systemically, resulting in limited cell killing at the tumor site due to a low concentration of the agent at the site as well as low uptake of the agent by tumor cells. INT230-6, designed for intratumoral administration for maximal uptake by cancer cells, is a combination of cisplatin and vinblastine and an amphiphilic cell penetration enhancer to improve cell uptake by diffusion.

Aim:

Determine if INT230-6 induces a strong and long lasting anti-tumor immunity.

Hypothesis I:

INT230-6 induces clinical response in established bulky tumors.

Results I:

INT230-6 had an clinical effect against Colon26 tumors with 100% regression from baseline and up to 80% CR.

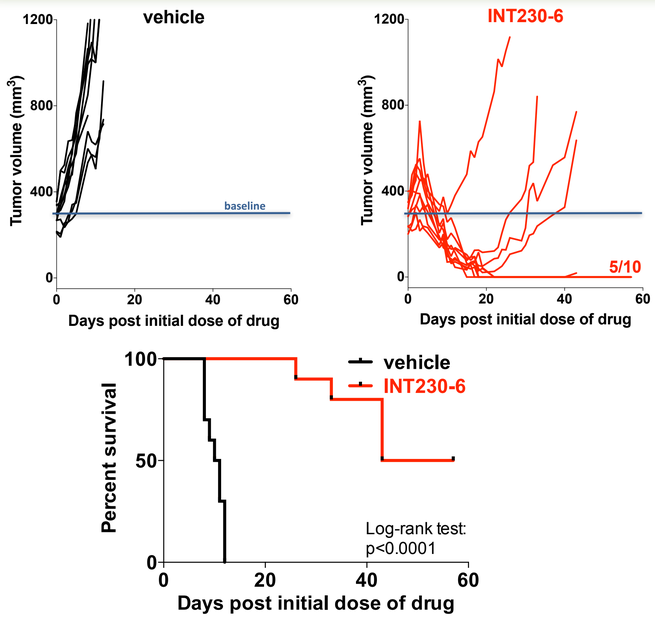

Figure 1. Vehicle or INT230-6 (100µl/400mm^3 tumor) were administrated intratumorally into 300mm^3 (approx. 9mm in diameter), subcutaneous Colon26 (C26) tumors for 5 sequential days (day 0 to 4). Kaplan-Meyer plot and individual responses are shown of vehicle (black) and INT230-6 (red) treatment. INT230-6 treatment resulted in prolonged survival, regression from baseline and up to 80% complete response (CR) (example of 50% CR shown).

Hypothesis II:

The efficacy of INT230-6 is dependent on the immune system.

Results II:

1. INT230-6 efficacy was dependent on CD8$^+$ T cells.

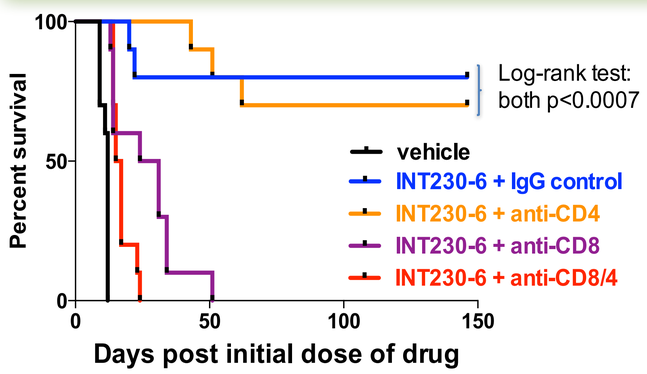

Figure 2. IgG control antibody (300µg), CD4 (100µg) and CD8 (200µg) depletion antibodies were administrated intraperitoneally (IP) on day 0, 1, 5, 8 and 15 post initial INT230-6 dose. Log-rank test was significantly different between INT230-6 + IgG control vs INT230-6 + anti-CD8 and INT230-6 + anti-CD8/4.

2. Mice with complete response (CR) were re-challenged and protected from tumor re-establishment in a T-cell-dependent manner suggesting immunological cell memory was induced by INT230-6.

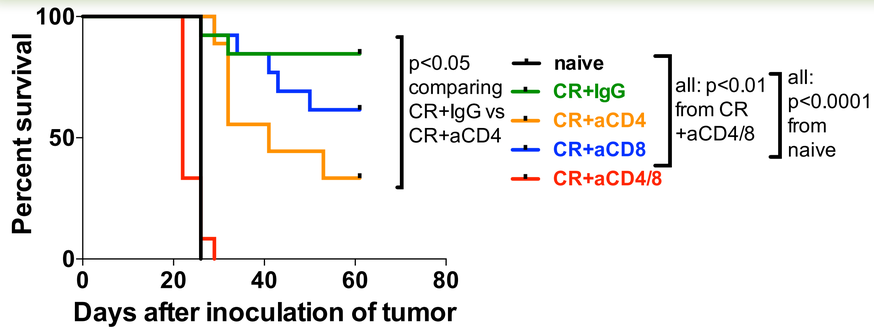

Figure 3. Mice with CR and naïve mice (black) were challenged with Colon26 by subcutaneous injection. Different groups of mice with CR including those with depletion of CD4 (yellow) and/or CD8 (red/blue) or control IgG (green, 150µg anti-CD8/4, 300µg IgG, day -1, 3, 5, 12 and 19) administrated IP were compared. Mice with CR+IgG (controls) were protected from tumor growth upon rechallenge. Depletion of CD4 T cells resulted in significant reduction in protection (3/9 mice vs 11/13 mice in CR+IgG group); however all mice lost protection upon double depletion only.

Figure 4. Colon26 cells were injected intravenously to produce lung or other metastases into complete responders (red) and naïve mice (black). Log-rank test was significantly different (p<0.0001) between the groups suggesting that immunization by INT230-6 protected from tumor metastasis formation.

3. INT230-6 induces CD8 T cell specific responses to tumor cells.

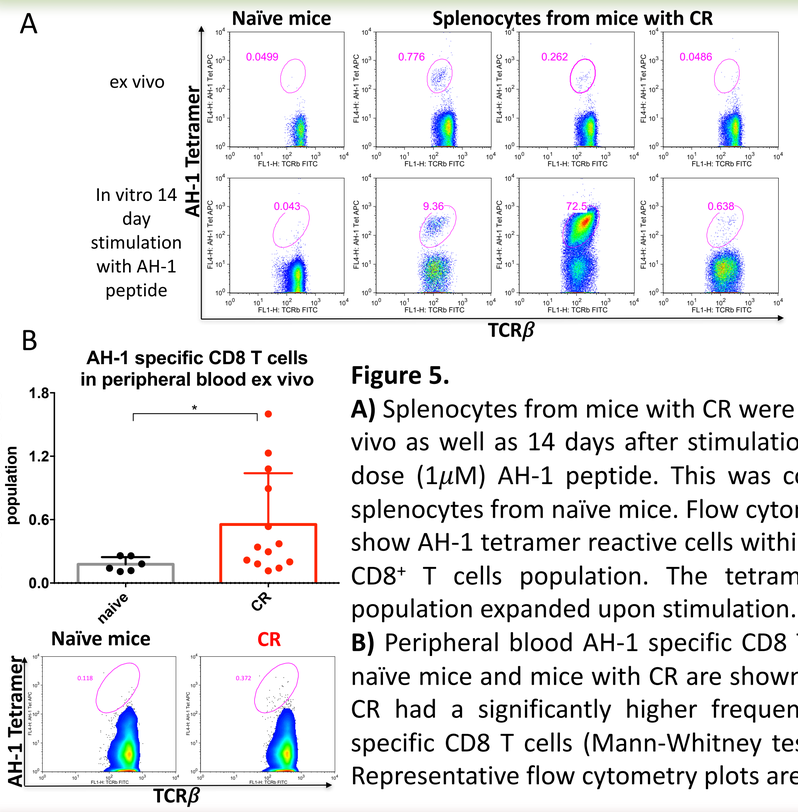

Figure 5.

A) Splenocytes from mice with CR were analyzed ex vivo as well as 14 days after stimulation with high dose (1μM) AH-1 peptide. This was compared to splenocytes from naïve mice. Flow cytometry plots show AH-1 tetramer reactive cells within the TCRβ^+ CD8$^+$ T cells population. The tetramer reactive population expanded upon stimulation.

B) Peripheral blood AH-1 specific CD8 T cells from naïve mice and mice with CR are shown. Mice with CR had a significantly higher frequency of AH-1 specific CD8 T cells (Mann-Whitney test *p<0.05). Representative flow cytometry plots are displayed.

Conclusion:

INT230-6 given locally to treat tumors induces tumor specific protective T cell immunity and clinical trials to test efficacy in cancer patients will be commencing soon.