A novel immunotherapy, INT230-6, is able to induce high rates of complete and durable response in mice through a cytotoxic T-cell-dependent mechanism

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Summary

The National Cancer Institute's Vaccine Branch and Intensity Therapeutics are collaborating to examine the immunologic mechanism of action of INT230-6, which consists of potent cytotoxic agents formulated with compounds that improve intracellular and tissue diffusion.

A key component to initiating a tumor immune response is priming the immune system with a broad range of tumor antigens. INT230-6 induces immunologic cell death locally in the tumor which recruits inflammatory cells and stimulates a potent systemic T-cell response.

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INTRODUCTION

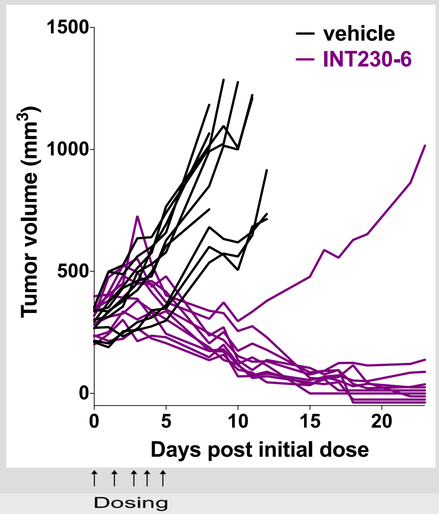
INT230-6 is a combination of cisplatin, vinblastine and an amphiphilic cell penetration excipient that when administered intratumorally (IT) can induce complete regression in drug-injected and bystander large colon26 (C26) mouse tumors.

INT230-6 also has the ability to induce long term protection in mice against intravenous (IV) or subcutaneous (SC) re-inoculation of the cancer cells.

Previous studies had not elucidated whether the drug-injected tumor regression in immuno-competent mice subcutaneously (SC) inoculated with Colon26 cancer was due to the formulation's direct cytotoxicity, or an immune-based cell response or a combination of both. Previous studies reported by our groups indicate that the dying cancer cells can serve as a vaccine.

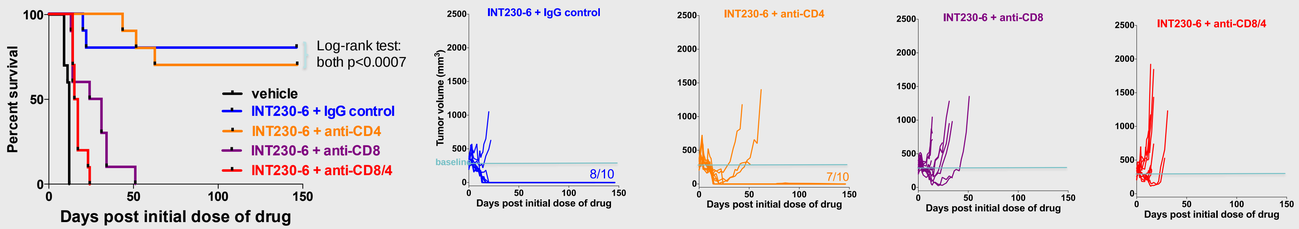
THE TIMECOURSE FOR TUMOR RESPONSE

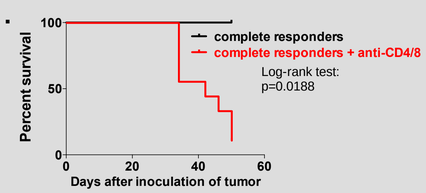
We conducted studies using 10 female syngeneic BALB/c mice per group inoculated SC with $1x10^6$ C26 carcinoma cells. Tumors grew to mean volumes of $300mm^3$ pre-dose. Animals received 0.1 mL of INT230-6 per $400mm^3$ of tumor volume for 5 days. There was no adjustment for body weight. Tumors all regress from baseline and continue to shrink well past the half life of the drugs



CD8⁺ T CELL DEPLETION REDUCED EFFICACY OF INT230-6 TREATMENT

CD4 and CD8 T cells were depleted at the onset of INT230-6 treatment and confirmed with flow cytometry. Vehicle control (**black**) resulted in short term survival. INT230-6 treatment combined with an IgG control antibody (blue) induced CR's in 80% of mice. Interestingly, depletion of CD4 T cells did not significantly alter the effect (yellow). Depletion of CD8 T cells (purple) significantly shortened survival; even though transient regression from baseline was observed in the majority of animals, no complete response was obtained. This loss of immunity was further amplified by simultaneous depletion of both $CD8^+$ and $CD4^+$ T cells (red). This suggests the **initial reduction in tumor mass was due to the cytotoxic effect of the cytotoxic agents but complete response was dependent on recruitment of primarily effector CD8 T cells.**

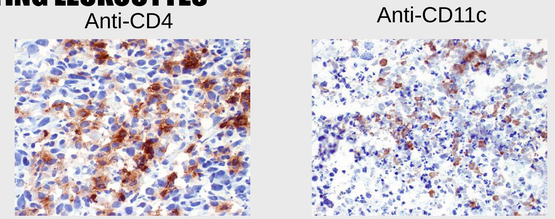




To look at long term memory, mice that were originally inoculated with C26 cells SC, treated with INT230-6 and achieved a CR, were re-challenged SC. CR mice re-inoculated with C26 spontaneously cleared the tumour cells. Depletion of both CD4 and CD8 T cells prior to re-challenge resulted in loss of protection evidenced by shortened survival and exponential tumor growth. **This indicated a CD4 and CD8 dependent immunological cell memory was generated by INT230-6.**

RECRUITMENT OF TUMOR INFILTRATING LEUKOCYTES

Biopsies of treated tumors confirmed rapid cell death with 75% of the tumor becoming necrotic in 3 days. By day 10, an increased influx of dendritic and T-cells was measured compared to controls. **This treatment can inflame tumors, presumably to process the large amount of tumor antigens released from the dying tumor.**



Images are taken at 40X magnification

DISCUSSION & CONCLUSIONS

Intratumoral administration of INT230-6, in addition to improving the direct cytotoxicity of cisplatin and vinblastine, induces a potent CD8 tumor-specific T-cell response which participates in regression of the injected lesion. CD4 help is needed to induce the long term memory response which protects animals from re-inoculation. This program is progressing towards the clinic.