Comparison of Anti-HIV ADC and Immunotoxin Showed Clinically Relevant Differences in Cytotoxic Effects

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BACKGROUND

The persistent reservoir of cells carrying a functional provirus is the barrier to HIV eradication. One approach to reservoir depletion is sequential activation of latent virus, followed by purging of HIV-expressing cells. We have armed antibodies with cytotoxic agents to enhance killing of HIV-infected cells, independent of ADCC or complement activity. We screened >200 mAbs to identify those most effective at delivering the toxic moiety, and found that the anti-gp41 mAb 7B2, conjugated to ricin A chain, is safe and effective in SHIV-infected macaques. However, it was immunogenic, limiting its utility. We therefore seek to develop less immunogenic immunotoxins (ITs) and antibody drug conjugates (ADCs).

METHODS

- We screened drugs for cytotoxicity on resting lymphocytes.
- ADCs were prepared by chemical conjugation to 7B2.
- Cytotoxicity of ADCs was compared to that of ITs on Env-expressing cells.
- The effect of ADCs and ITs on metabolism and transcription in HIV-infected cells was studied by NMR-based metabolomics and RNAseq.

Cytotoxic immunoconjugates targeted by anti-HIV mAbs have demonstrated potent anti-viral effects in vitro and in vivo.

Ricin toxin kills non-dividing lymphocytes, as does the small drug PNU-159682. Anti-gp41 mab 7B2 was conjugated to ricin A chain (dgA) and to PNU, creating an immunotoxin and ADC which were then compared.

7B2-dgA killed cells at lower drug concentrations and more rapidly, but only 7B2-PNU demonstrated bystander cell killing.

The IT and ADC initiated distinctly different metabolic and transcriptional states in killing HIV-infected cells.

The differences in modes of killing may be of potential clinical relevance.

CONCLUSIONS

- Cytotoxic immunoconjugates targeted by anti-gp41 mAb 7B2 were effective at clinically achievable concentrations.
- The persistent reservoir of HIV infection may contain non-dividing cells. Both ricin and PNU killed resting lymphocytes.
- 7B2-dgA killed more potently and rapidly than 7B2-PNU.
- 7B2-immunoconjugates killed Env+ cells at a 30-1000-fold lower concentrations than non-specifically kill Env- cells.
- 7B2-PNU caused bystander cell killing. Therapeutically this could lead to either better efficacy or unwanted side-effects.
- Metabolic profiling of cells following treatment revealed significant differences between 7B2-dgA and 7B2-PNU, with the former leading to increased intracellular AA pools, but decreased creatine, fumarate, and taurine within 6 hr.
- Transcriptional profiling by RNAseq also showed marked differences between types of treatment, with 7B2-dgA initiating changes within 6 hr including marked upregulation of apoptosis and transcriptional regulatory pathways. At 24 hr 7B2-dgA treated cells down regulated AA biosynthesis.
- The differences in the mechanisms of killing by ADCs and immunotoxins may have clinical relevance.

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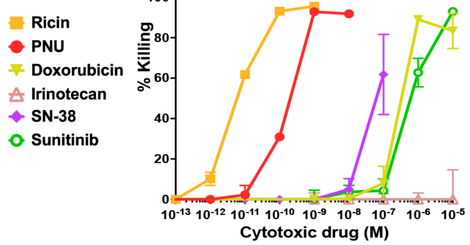
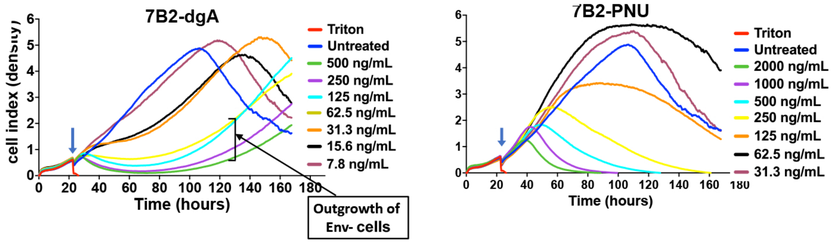
RESULTS

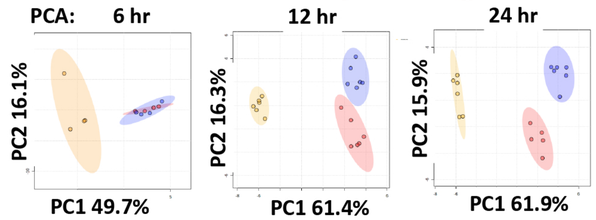
Ricin and PNU kill resting lymphocytes

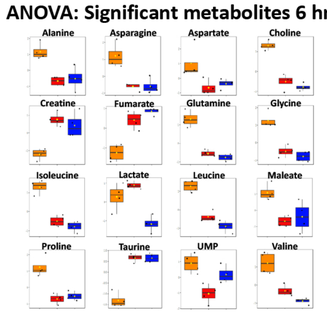


Kinetics of killing



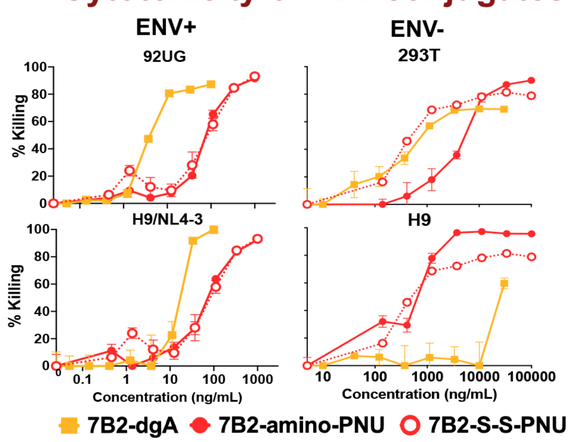
Metabolic profiling



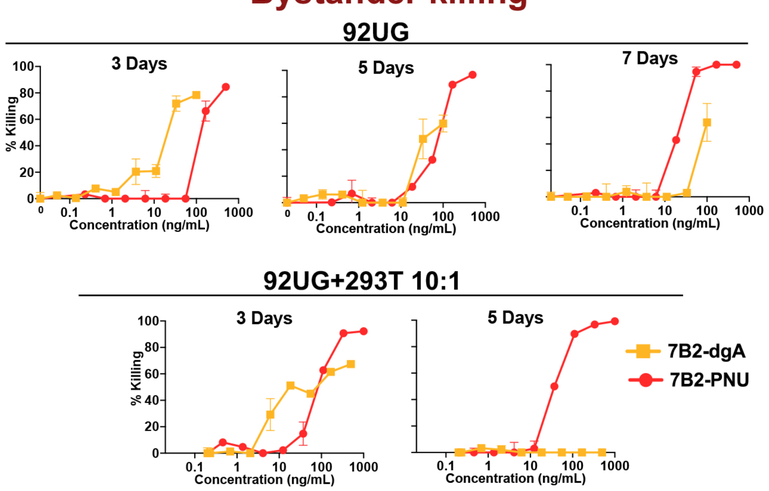


KEY: Untreated 7B2-dgA 7B2-PNU

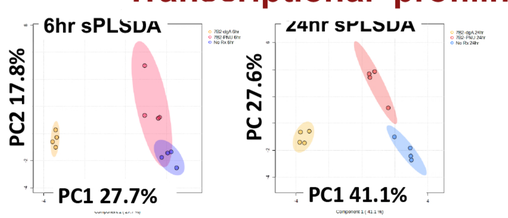
Cytotoxicity of 7B2 conjugates



Bystander killing



Transcriptional profiling by RNAseq



Differentially Regulated Genes (of 8617 total)

Treatment	Time	Up*	Down*
Untreated vs 7B2-dgA	6 hr	347	20
Untreated vs 7B2-PNU	6 hr	0	0
7B2-dgA vs 7B2-PNU	6 hr	12	158
Untreated vs 7B2-dgA	24 hr	1487	821
Untreated vs 7B2-PNU	24 hr	867	287
7B2-dgA vs 7B2-PNU	24 hr	567	637

*Genes with adjusted p value <0.05 AND [log2] fold change >1

Gene Pathway Analysis

