DPX-based immune education recruits and activates unique subsets of antigen presenting cells to drive immunogenicity of peptide antigens

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ABSTRACT & BACKGROUND

For peptide-based cancer vaccines, successful eradication of tumors relies on the effective and persistent delivery of antigenic peptides to antigen presenting cells (APCs) to prime potent antigen-specific, cytotoxic T cells. Peptide-based vaccines have historically been either; (1) water-based formulations, which provide short exposure of peptides to immune cells; or (2) oil-in-water emulsions that provide longer peptide exposure, but can elicit dysfunctional and exhausted T cell phenotypes. By contrast, the DPX® technology is a non-aqueous, lipid-in-oil, immune-educating therapeutic delivery platform. Antigenic peptides formulated in DPX elicit a robust, targeted, and persistent tumor antigen-specific T cell response that for our lead DPX product, Maveropepimut-S (MVP-S), has translated into clinical benefit in multiple cancer indications, including DLBCL and ovarian cancer.
Herein, we compare mechanisms of immune activation amongst DPX, aqueous and emulsion-based formulations.

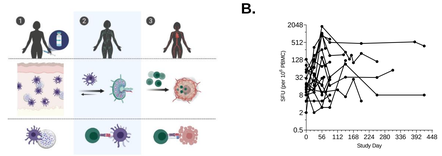
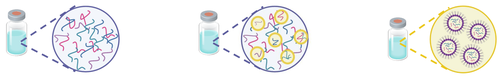


Figure 1: MVP-S is a DPX formulation that incorporates 5 HLA-restricted peptides derived from the anti-apoptotic protein survivin, which is commonly overexpressed in advanced cancers. (A) Mechanism of action of MVP-S (1) Injected MVP-S is actively engulfed by antigen-presenting cells (APCs). (2) APCs traffic to lymph nodes to activate survivin-specific T cells. (3) survivin-specific T cells traffic to target survivin-expressing tumor cells. **(B) *De novo* activation of durable and robust survivin-specific T cells in response to MVP-S immunotherapy.** Increases in antigen-specific T cells were detected in PBMCs of nearly 2/3 of all evaluable subjects by *ex vivo* IFN-γ ELISPOT (*Dorigo et al. 2022*).

In contrast, *Lennerz et al. 2014* demonstrated that the same survivin peptides formulated in standard Montanide-based emulsion elicited positive *ex vivo* ELISPOT responses in just 14% of the patients with advanced solid tumors.

MATERIALS & METHODS

Formulations: longitudinal analysis of the site of injection (SOI; Figure 2A) was conducted using $OVA_{323-339}$ peptide formulated in DPX. All other experiments were performed using formulations containing $OVA_{257-264}$ peptide and polynucleotide adjuvant. Empty formulations contained no peptide or adjuvant, but all other components and method of formulation were as for the corresponding peptide formulations.

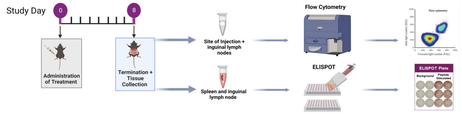


Aqueous Delivery
(peptides/adjuvant solubilized in aqueous buffer)

Emulsion Delivery
(delivery of hydrophilic cargo in buffer and hydrophobic cargo in oil)

DPX Delivery
(single phase formulation composed of lipid reverse micelle with peptides and adjuvant resuspended in oil)

Experiment Design: immune cell composition and antigen uptake at the SOI were assessed by multi-parameter flow cytometry. Antigen-specific immune responses were assessed in draining lymph nodes and/ spleens by IFN-γ ELISPOT.



RESULTS

Aqueous formulations fail to elicit substantive, antigen-specific immune responses

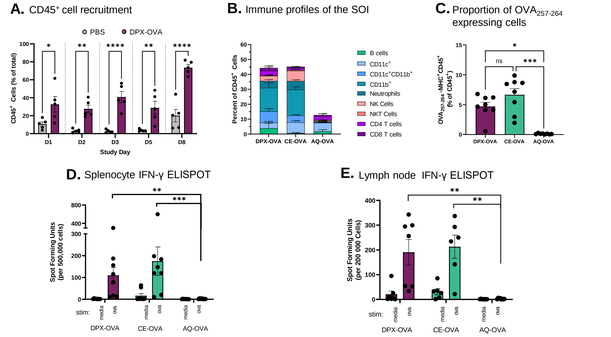


Figure 2: Comparison of immune responses driven by aqueous formulations (AQ), conventional emulsions (CE) or DPX. **(A)** Frequency of lymphocytes (CD45⁺ cells) recruited to the site of injection (SOI) 1, 2, 3, 5 and 8 days post $DPX\text{-}OVA_{323-339}$ treatment; **(B)** comparison of immune profiles at SOI 8 days post administration of DPX- $OVA_{257-264}$, CE- $OVA_{257-264}$ or Aq-$OVA_{257-264}$; **(C)** frequency of lymphocytes that express $OVA_{257-264}$ on MHC-I at the SOI 8 days post treatment with DPX- $OVA_{257-264}$, CE- $OVA_{257-264}$ or Aq-$OVA_{257-264}$; **(A-C)** are quantified by flow cytometry. **(D-E)** $OVA_{257-264}$-specific immune responses in the spleen and treatment-draining lymph nodes 8 days post administration of DPX- $OVA_{257-264}$, CE- $OVA_{257-264}$ or Aq-$OVA_{257-264}$ assessed by IFN-γ ELISPOT. Data are presented as spot forming units per 500,00cells and 200,000 cells respectively. * p<0.05,**p<0.01, ***p<0.0001.

The DPX platform has unique intrinsic properties which allows for the recruitment and activation $CD11b^+CD11c^+$ cells independent of peptide presence in the formulation

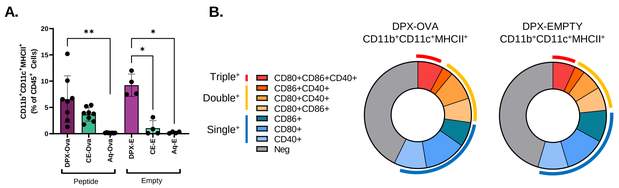


Figure 4: Analysis of immune infiltration at SOI 8 days after administration of DPX-Empty (DPX-E), CE-Empty (CE-E) or Aq-Empty (Aq-E). Empty formulations contain no peptide or an adjuvant. **(A)** frequency of $CD11b^+CD11c^+MHCII^+$ cells within lymphocyte population at SOI quantified by flow cytometry; **(B)** comparison of frequencies of $CD11b^+CD11c^+MHCII^+$ cells that co-express activation/stimulatory markers between groups treated with DPX-$OVA_{257-264}$ and with DPX-Empty. * p<0.05,**p<0.01.

Recruitment of the $CD11b^+CD11c^+$ subset to the SOI of CE is dependent on the peptide presence in CE. In contrast, for DPX, the recruitment and activation of $CD11b^+CD11c^+$ subset is evident regardless of whether a peptide is present. This suggests that DPX platform has unique intrinsic ability to recruit specific subsets of APCs.

Compared to conventional emulsion, DPX platform recruits distinct subsets of antigen presenting cells with high propensity for activation

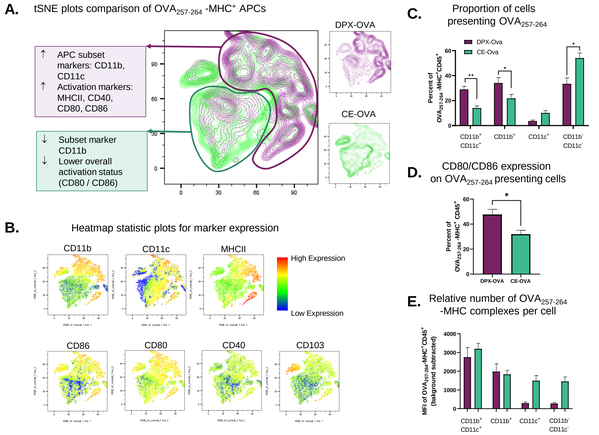


Figure 3: Comparison of antigen presenting cells attracted by DPX-$OVA_{257-264}$ to those attracted by CE-$OVA_{257-264}$. SOI profiles 8 days post treatment analyzed by multiparametric flow cytometry. **(A)** tSNE analysis of lymphocytes expressing $OVA_{257-264}$ on MHC-I; contour map of $CD45^{OVA\text{-}MHC}$ induced by DPX-$OVA_{257-264}$ in purple, and contour map of $CD45^{OVA\text{-}MHC}$ induced by CE-$OVA_{257-264}$ in green; **(B)** heatmap statistics are represented in dot plots with red indicating high expression, and blue indicating low expression of indicated marker; **(C)** phenotypes and frequencies of $OVA_{257-264}$ presenting cells within lymphocyte ($CD45^+$) population; **(D)** expression of activation and co-stimulatory markers (CD80 and/or CD86) on cells expressing $OVA_{257-264}$–MHC; **(E)** relative numbers of $OVA_{257-264}$-MHC complexes per cell as determined by mean fluorescence intensity (MFI) for different subtypes of antigen presenting cells; results shown after subtracting background. * p<0.05,**p<0.01.

Antigen presentation driven by the DPX platform and by CE formulation shows different immune profiles. The DPX-OVA formulation delivers a substantial fraction of the peptide to the subset of $CD11b^+CD11c^+$ cells enriched in activation and co-stimulatory markers. In contrast, CE-OVA recruits a $CD11b^-CD11c^-$ subset. $CD11b^+CD11c^+$ cells have tendency to express higher number of $OVA_{257-264}$-MHC complexes per cell compared to the $CD11b^-CD11c^-$ population (Fig. 3E).

Recruitment of activated $CD11b^+CD11c^+$ cells to the draining lymph nodes is elevated with DPX delivery

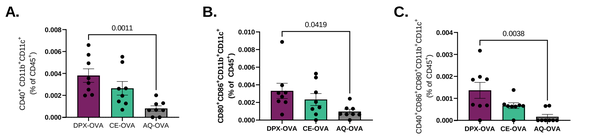


Figure 5: Comparison of frequencies of different subsets of $CD11b^+CD11c^+$ cells in the draining lymph nodes 8 days after administration of DPX- $OVA_{257-264}$, CE- $OVA_{257-264}$ or Aq-$OVA_{257-264}$. Indicated populations are expressed as frequency of total $CD45^+$ population.

$CD11b^+CD11c^+$ APCs co-expressing the CD80, CD86, and CD40 activation/costimulatory markers are enriched in the draining lymph nodes of DPX treated mice.

CONCLUSION

DPX is a unique, water-free, lipid-in-oil formulation that can deliver peptides to induce targeted, robust, and sustained immune responses. The unique nature of the lipid-in-oil DPX formulation keeps immune educating cargo at the site of injection, ensuring that uptake of the DPX encapsulated cargo is an active process driven by antigen presenting cells across time. In this manner, DPX controls the flow of immune educating cargo through specific APCs and into regional lymph nodes. By contrast, emulsion-based and aqueous formulations leach cargo into surrounding tissues allowing for passive and active immune cell engagement. With this presentation, we now show that:

- Aqueous formulations were poorly able to elicit a detectable IFN-γ ELISPOT response. Formulations containing an oil component (DPX and CE) were superior in recruiting APCs to the SOIs and inducing antigen-specific immune responses.
- Antigen presentation driven by the DPX platform had a distinct profile and was enriched in $CD11b^+CD11c^+MHCII^+$ APCs co-expressing the CD80, CD86, and CD40 activation/costimulatory markers known to be important for T cell activation. By contrast, the emulsion incited a distinctly different, $CD11b^-CD11c^-$ population.
- Recruitment and activation of a $CD11b^+CD11c^+$ subset is one of the hallmarks of the DPX platform and is independent of packaged cargo.
- Similar to the enrichment at the site of DPX injection with activated $CD11b^+CD11c^+$, this population was also elevated in the DPX draining LNs.
- Functional analysis of the $CD11b^+CD11c^+$ subset and its specific contribution to the quality and quantity of T cell responses is ongoing.

Collectively these findings highlight quantitative and qualitative differences in immune cell recruitment amongst three delivery platforms and show the unique character of the immune response triggered by the DPX platform typified by the recruitment of $CD11b^+CD11c^+$ APCs that have intrinsically higher capacity for antigen uptake, presentation, and activation.

FURTHER INFORMATION

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Literature cited: Lennerz V, Gross S, Gallerani E, *et al.* Immunologic response to the survivin-derived multi-epitope vaccine EMD640744 in patients with advanced solid tumors. Cancer Immunol Immunother. 2014;63(4):381-394. doi:10.1007/s00262-013-1516-5

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