Background Type-1 Interferons (IFN) are known to elicit both direct antitumor effects as well as modulate tumor microenvironment (TME) to induce antitumor immune responses. Combination of IFNa with PD-1 blockade has shown promising outcomes in patients with melanoma. However, clinical use of currently marketed IFNa products is limited due to poor systemic tolerability. Using our breakthrough XpressCF+™ cell-free technology we have developed a conditionally active IFNa-2b prodrug designed to widen the therapeutic window by virtue of limited systemic activation and preferential tumor-selective activation.

Methods IFNa-2b was prodrugged via site-specific conjugation of releasable polyethylene glycol (PEG) molecules, enabling both half-life extension (HLE) and tumor-selective demasking of the interferon molecules. PEG molecules were attached via a tumor selective linker, taking advantage of specific protease enrichment in the TME across a broad range of cancer indications. The size of PEG and sites of conjugation were chosen to enable an optimal balance of HLE, prodrug attenuation, demasking and potency of released catabolite.

Results In-vitro human IFNa-2b prodrug (IFNa-2b-prodrug) was greater than 1000-fold attenuated compared to recombinant IFNa-2b, thus supporting reduced systemic activation. However, on release of PEG activity of the catabolite is fully restored and comparable to wild-type protein. Site-specific PEG conjugation also conferred significant HLE supporting less frequent systemic administration. In mouse xenograft model MDA-MB-231 grown in immunocompromised mice, IFNa-2b-prodrug induced greater antitumor activity compared to control IFNa-2b variant with a non-releasable PEG mask. At equivalent doses, antitumor activity of the prodrug was significantly greater in mice engrafted with human peripheral blood mononuclear cells. This suggests IFNa-2b-prodrug elicits antitumor response both via direct action on tumor cells and by engaging the immune system. Mouse surrogate for IFNa-2b-prodrug induced potent tumor growth inhibition in both immunogenic and less immunogenic syngeneic tumor models. Response in syngeneic models was associated with increased expression of cytotoxic effector molecules in TME. Finally, we used hamsters to assess tolerability and showed that single dose of IFNa-2b-prodrug is well tolerated up to 45 mg/kg with no body weight loss and minimal liver enzyme induction. In contrast, both HLE-IFNa-2b variant and IFNa-2b-prodrug masked with a more permissible PEG linkage, exhibited poor tolerability with significant systemic activation.

Conclusions In summary, these preclinical data suggest this novel HLE, tumor-selective human IFNa-2b prodrug has the potential to improve therapeutic index of IFNa therapies. Moreover, the results also support further development of this molecule as a single agent and in a combination setting.