Abstract
While whole-HER2 and HER-2 directed therapy is active and well-tolerated across solid tumor malignancies, studies investigating novel agents and combination therapies with HER-2 directed therapy, are also under investigation. One strategy of interest has been investigating the potential therapeutic role of HER-2 peptide cancer vaccines, which has the potential to result in a sustained immunological tumor response.
We have advanced a new paradigm in immunotherapy that focuses on humoral responses based on conventional B-cell epitope vaccines. These novel platforms elicit high affinity anti-epitope antibodies against tumors that help circumvent intrinsic drug resistance. Preclinically, we identified the first generation of HER-2 B-cell epitope peptide and through computer immunogenicity algorithms and extensive in vitro and in vivo preclinical studies. In a recently published phase 1 clinical trial, we showed that the combination of the two chimeric HER-2 vaccine in patients with metastatic solid tumors was safe, demonstrated activity (disease control rate 25%), and elicited HER-2 specific humoral response in 25% of patients. Our group has developed two novel B-cell epitope specific vaccines consisting of epitope derived from the extracellular domain of the HER-2/neu molecule that are currently being tested in early clinical trials for breast and colorectal tumors.
In extensive preclinical studies, we have demonstrated that the two engineered epitopes mimicked the three-dimensional structure of the HER-2/neu receptor and elicited high affinity antibodies that recognized the native HER2.
Additionally, these peptides inhibited multiple signaling pathways including HER-2 specific inhibition of cellular proliferation and phosphorylation of downstream molecules. These molecules were validated in vivo in a xenograft model of metastatic breast cancer under conditions of intravenous and subcutaneous administration.
These vaccines had statistically reduced tumor mass in two transplantable tumor models (MDA and B16C10) and led to tumor regression in a xenograft model of breast cancer. These two vaccines exhibited properties similar to trastuzumab and pertuzumab, validating their use in the phase 1 clinical trial.
Peptide vaccines offer several advantages over the current state of cancer with combination immunotherapies, including increased safety, cost-effectiveness and ease of administration. Additional advantages of active cancer vaccines are exquisite specificity, low toxicity, and the potential for long-lasting immunity.
Herein, we report the results from the first human, dose escalation portion of the phase Ib study testing the combination of two B-cell epitope vaccine MFV-HER-2 (63-76) and MFV-HER-2 [263-264] incorporating a promiscuous measles virus (MV) T-cell epitope engine to target the trastuzumab and pertuzumab binding sites.
Objectives
The primary objectives were to assess safety and clinical toxicity of immunization, determine the optimal immunogenicity drug dose (CIBD) of combination HER-2 vaccines, measure both humoral and cellular immune responses including the specificity, class, and kinetics of anti-HER-2 peptide secondary objectives were to collect and analyze samples and peripheral blood cells for additional studies following the last injection and document clinical responses.
Evaluate whether the combination of HER-2 vaccines demonstrate therapeutic benefit, provide synergistic and/or additive effects, and to enumerate mechanisms of action.
Study design and methods
The GMP peptides were purchased from Peptherapy (Tonawanda, NY, CA) and acquired by Sloepue Group (Zug, Switzerland). The GMP peptides met all the FDA and US Pharmacopeia requirements for identity (ie, homogeneity), contaminants, and potency. ELISA titers of these peptides were tested and determined to be within acceptable levels as (GMP) grade.
The combination vaccine MFV-HER-2 (63-76) and MFV-HER-2 [263-264] were produced in a two step process involving two separate reactors. The fusion protein was expressed in bacteria (aCella, Saab T, and Kaumaya, PTP) and purified overexpression (MVF) was performed through a mammalian cell expression system. The protein was purified from the baculovirus (MVF) and was used in the phase I trials (Penninsula Labs, Tonawanda, NY).
The vehicle Montanide ISA (72) was purchased from SEPPIC (Paris, France) and it had an approval certificate of analysis for toxicity, emulating property exactly.
The immunogenicity of each individual peptide and combination was verified in pairs of New Zealand rabbits.
The combination vaccine was administered and monitored to two HER-2 B-cell epitope peptides with MV-HPD adjacent (02535G) in a total volume of 1.0 mL and emulsified 1 mL with ISA 720.

The assessment time (per patient) for injection was as follows:

- 0 (0%)
- 1 (2%)
- 2 (3%)
- 3 (7%)
- 4 (12%)
- 5 (20%)
- 6 (40%)
- 7 (20%)
- 8 (12%)
- 9 (3%)
- 10 (2%)

- 6 patients at each dose level were required to receive 3 inoculations of the combination vaccines at 3 week intervals.
- Injections occurred at the following time points: days 1, 12, 23, 34, 45, 56, 67, 78.
- Injections were given at the dose level and the dose level was observed for a minimum of 6 weeks. Dose escalation could proceed if no serious adverse reactions or dose-limiting toxicities were observed.
- The vaccine was well tolerated with dose level 2 defined as the CIBD/OID and as the recommended phase II dose.
- The most common related toxicity in all patients was injection site reactions (24%).
- The combination vaccine showed effectiveness in eliciting antibody responses without serious adverse reactions, treatment discontinuation due to unacceptable DLTs, or evidence of treatment resistance.
- The vaccine is safe, exhibits anti-tumor activity and shows preliminary indication that peptide vaccination may yield therapeutic resistance and offer a promising alternative to monoclonal antibody therapies.

Conclusions

- Forty-nine patients with a median of 4 prior lines of chemotherapy receiving at least 1 vaccination. Twenty-eight patients completed the 3 vaccination regimen. Six patients received 1 vaccine (1 month after the first dose) and 7 received 1 vaccination after 7 months. No serious adverse reactions or dose-limiting toxicities were observed.
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- The most common related toxicity in all patients was injection site reactions (24%).
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- The vaccine is safe, exhibits anti-tumor activity and shows preliminary indication that peptide vaccination may yield therapeutic resistance and offer a promising alternative to monoclonal antibody therapies.

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References

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Figure 1. Western Blots image of 4 patients stained with patient sera and used to determine the epitopes used in the vaccine. Each lane contains a 10% SDS-PAGE gel, stained with Coomassie blue. The antibody used was against total HER2 protein.

Figure 2. Immunohistochemical staining of HER2 tumor samples treated with the patient sera used for the western blots. Each lane contains a 10% SDS-PAGE gel, stained with Coomassie blue. The antibody used was against total HER2 protein.

Figure 3. Cohort 2 patients elicited native HER-2 IgG (B174T) and recombinant HER-2 anti-epitope antibodies.

Table 1. Summary of patients in cohort 1 & 2

Table 2. Table 2. Treatment related toxicities

Figure 4. Patient 2C antibody binding over 7 boosters (3.5ears).

Figure 5. Effects of purified Abs on proliferation, phosphorylation, apoptosis, and ASCD