Development of a Quadrivalent Peptide Lynch Syndrome Vaccine

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Abstract

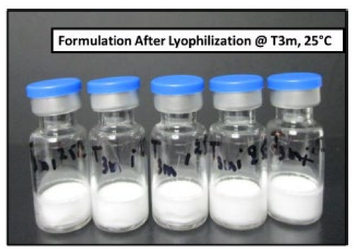
Some cancers are deficient in their ability to repair mismatched DNA which results in the accumulation of frameshift mutations in genes that contain microsatellite sequences, repeated sequences of DNA. Translation of these genes generates proteins with frameshift peptides induced by the mutations. These neoantigen peptides can provide a means for the immune system to target these microsatellite-instability (MSI) cancers. Such frameshift peptides have been identified in four proteins, TAFB(-1), AIM2(-1), HT001(-1) and TGFBR2(-1) are being developed as vaccines to prevent/treat colorectal cancer, endometrial cancer, gastric cancer or small bowel cancer. A stable lyophilized formulation containing quadrivalent frameshift peptides (FSP) AIM2(-1), HT001(-1), and TAFB (-1), TGFBR2(-1), was obtained through a systematic screening process. The screening included evaluation of pH, stabilizers, bulking agents, and surfactants. The screening resulted in a scalable formulation and lyophilization process with 100 µg/ml each peptide,10 mM Histidine at pH 5.5, 290 mM trehalose, and 0.02% polysorbate 20 as a clinical formulation. Based on peptide content and peptide purity from a three-month stability study, the lyophilized formulation is estimated to be stable at ambient temperatures for 18 months. Asymptomatic carriers of DNA mis-match repair mutations (Lynch Syndrome (LS) carriers) will be the target population to be vaccinated and monitored for safety and immunogenicity in a Phase 1 clinical study.

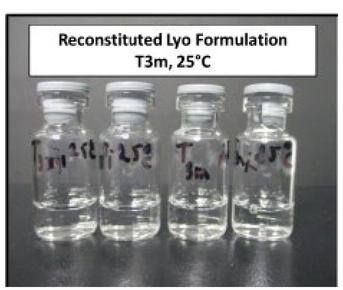
Methods and Materials

- Four peptide sequences were synthesized by PolyPeptide Laboratories, San Diego, CA
- A formulation containing all four peptides was developed using factorial design experiments by CuriRx.

Introduction

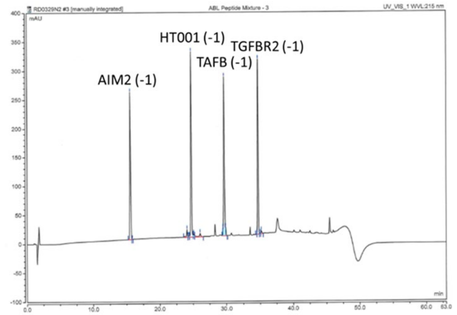
- One in 280 Americans estimated to have LS gene mutations. LS confers 70-80% lifetime risk of developing colorectal cancer (CRC) and is the most common CRC genetic syndrome.
- Lynch syndrome is an autosomal dominant cancer predisposition syndrome caused by germline mutations in the MLH1, MSH2, MSH6, PMS2or EPCAM gene, resulting in a defect in DNA mismatch repair. MMR leads to microsatellite instability. These MSI regions code frameshift peptide neoantigens.
- Four peptide sequences were identified and formulated as a vaccine to be used in patients with LS.
  - **TAFB(-1):** NRGLKKKTILKKAGIGMCVKVSSIFFIN KQKP
  - **AIM2(-1):** VIKAKKKHREVKRTNSSQLV
  - **HT001(-1):** RSNSKKKGRRNRIPAVLRTEGEPLHTP SVGMRETTGLGC
  - **TGFBR2(-1):** KEKKSLVRLSSCVPVALMSAMTT SSSQKNITPAILT (modified)



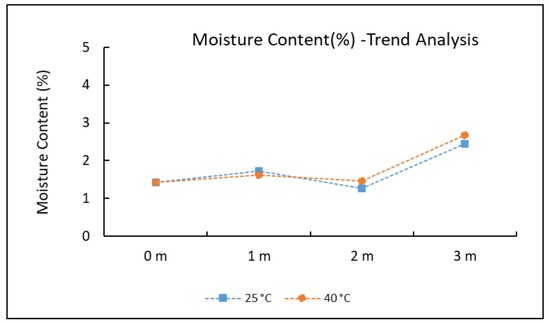


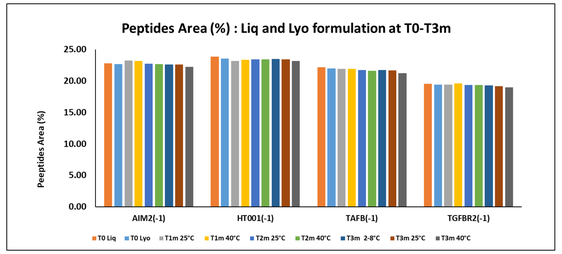
Formulation Development

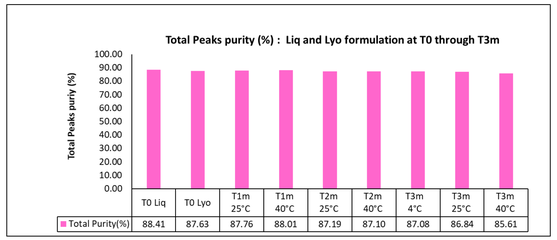
- The analysis of formulation conducted by RP-HPLC which was able to resolve all 4 individual peptides.



- The final formulation matrix consisted of **10mM Histidine, 290mM Trehalose, 0.02% Polysorbate 20, 0.1 mg/mL of each of the four peptide sequences.**
- The lyophilized vial contains 0.4 mg of peptide along with the excipients given above.
- An accelerated stability study of the peptides in formulation buffer was conducted and shown to be stable over 3 months.
- The final formulated quadrivalent vaccine is stable at refrigerated temperature for up to 3 months.







Analysis of Subvisible Particles before & after lyophilization

Particles Count (P/mL)									
Filter ID	T0 Liq	T0 Lyo	T1m 25° C	T1m 40° C	T2m 25° C	T2m 40° C	T3m 2-8° C	T3m 25° C	T3m 40° C
2-10 µm	461	997	1711	2425	646	1093	1605	2798	1415
10-25 µm	60	74	60	357	91	99	74	124	190
25-50 µm	0	0	0	45	8	25	8	8	0
50-100 µm	0	0	0	15	0	8	0	0	0
100+µm	0	0	0	0	0	0	0	0	0
Total Count (p/mL)	521	1071	1771	2842	745	1225	1687	2930	1605

Conclusions

- The four peptide sequences were identified from LS patients with MSI-H which code frameshift peptide neoantigens.
- The final formulation matrix consisted of **10mM Histidine, 290mM Trehalose, 0.02% Polysorbate 20, 0.1 mg/mL of each of the four peptide sequences**
- At T3m, the lyophilized cakes were white in appearance without any collapse or crack and fully plugged. The reconstituted liquid formulation was clear colorless and free from visible particles.
- The reconstitution time is less than 10 seconds through all time points.
- The pH and tonicity after lyophilization of 4 peptides were in the target average range of pH 5.59 and 308 mOsmo/kg.
- At T0 through 3 months, peptide area percent, peptide content and peptide amount per vial is similar for each of the four peptides with no significant changes.
- The total impurities percent was about 11.6 to 14.4% in reconstituted lyophilized formulation from T0 through T3m.
- Both liquid and lyophilized formulations from t0 through T3m 10 µm+ particles were <360 and did not increase with the time and temperature.
- The moisture content for the lyophilized product is less than 5%.
- The lyophilized formulation is stable for three months with no significant change in appearance, pH, peptide area percent, peptide contents and the total peptide impurities.

References

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2. Guo Y, Lei K, Tang L. 2018. Neoantigen vaccine delivery for personalized anticancer immunotherapy. Front. Immunol. 9:1499

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