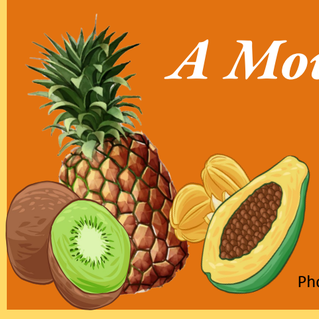
Model Validation and Preclinical Testing of Digestive Enzymes for Gluten Breakdown:

A Move to Cure Gluten Intolerance and Celiac Disease



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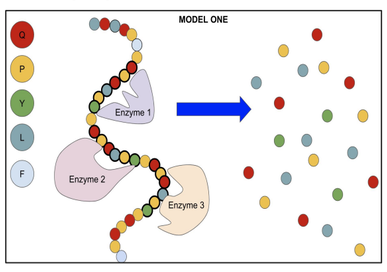
Background

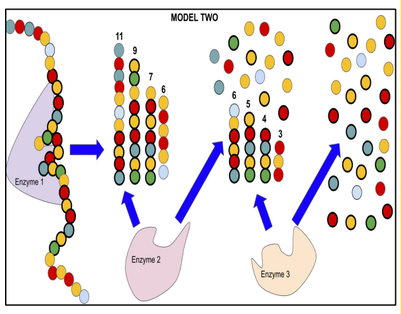
Celiac Disease and Gluten Intolerance

- *There is an unmet need to identify a solution for Celiac Disease and Gluten Intolerance*
- To meet this need, last year, I identified that three fruit enzymes Papain (in Papaya Skin), Bromelain (in Pineapple) and Actinidin (in Kiwi) when combined respectively in the 1:2:3 ratio can effectively breakdown gluten proteins in wheat. This combination of enzymes is referred to as **1-2-3 EC MIX.**
  - Based on this finding I proposed two models for the breakdown of gluten by the three enzymes.
- This year, I continued to validate my findings and my proposed models to move it one step closer to clinics as I am optimistic about the potential this novel enzyme mix holds for this patient population.



MODEL 1
Gluten Breakdown occurring because each enzyme targets different part of the Gliadin Molecule.

MODEL 2
Sequential Breaking Down of Gluten Molecule by the three enzymes



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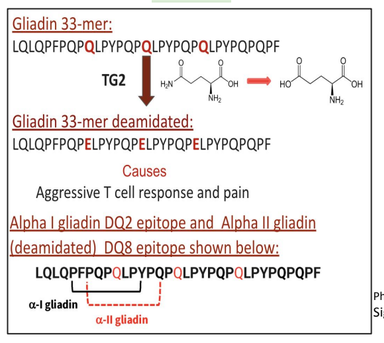
Rationale

- Previous studies have shown that.
  - A larger 33-mer peptide from gliadin is an immuno-dominant gluten peptide that initiates strong immune response in CD patients
  - This 33-mer gets deamidated by Transglutaminase (TG2) that causes severe T-cell immune stimulation.
  - To test my proposed models, this year I intend to use these peptides and investigate the efficacy of **1-2-3 EC MIX** in degrading the immunogenic peptides.

Immunogenic Peptides in Gluten



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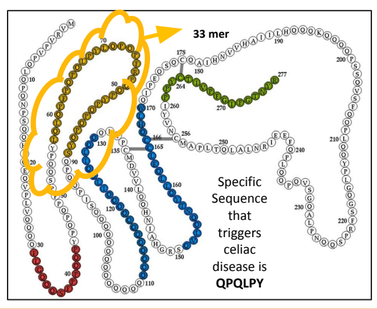
Gliadin Molecule



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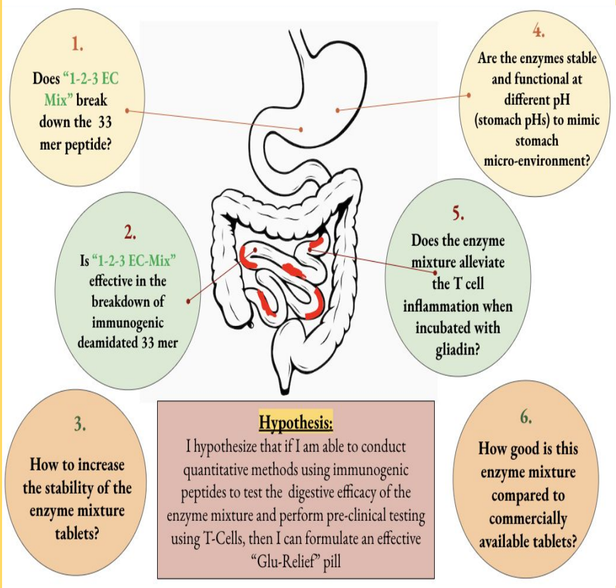
Research Question and Hypothesis



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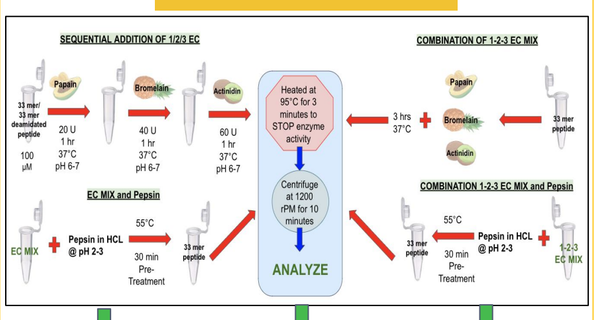
Experimental Methods

Gliadin peptides treatment with 1-2-3 EC MIX



Western and ELISA Assay | **Mass Spectrometry** | **Cytokine Assay and T-Cells**

Photo Credit: (Tube); Amazon.com, (Fruits); Slidesgo.com

Results

Mass Spectrometry Peaks and Identification of Samples

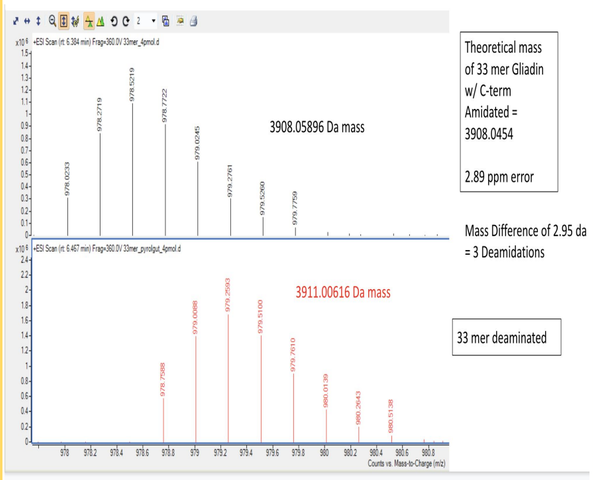


Figure 1: **(m/z peaks plot)**-This mass spectrometry data plots show the peaks corresponding to the 33 mer and the 33 mer deamidated peptides having a charge of 4. The first peak at 978.023 for 33 mer and 978.7588 are called the parent peak. The rest of the peak profile are the isotope(^{13}C) peak profile. Photo Credit: Aditi Avinash

Results 1- Digestion of the Immunodominant Gluten Peptides by 1-2-3 EC /MIX

Figure 2

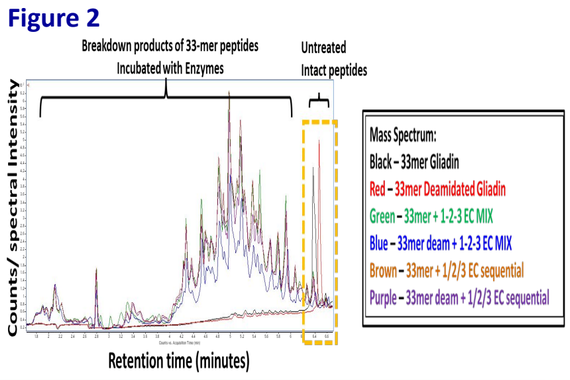


Figure 3

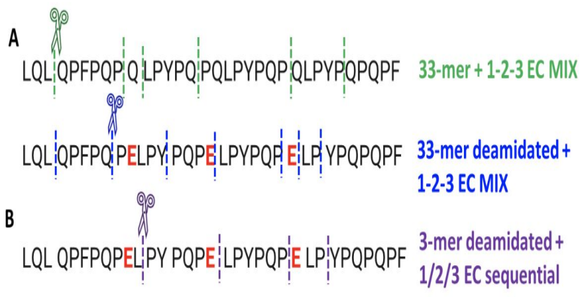


Figure 3: **Identification of cleaved Sites from the LC/MS and indicated by dotted lines. (A)** Cleaved sites of 33 –mer and 33-deamidated peptide with combination therapy. **(B)** Cleaved sites of 33-deamidated peptide with sequential monotherapy of 3 enzymes.

Photo Credit: Aditi Avinash

Results CONTINUED

Results 2- 1-2-3 EC MIX is effective in breaking down gliadin in the stomach acidic environment

Figure 4

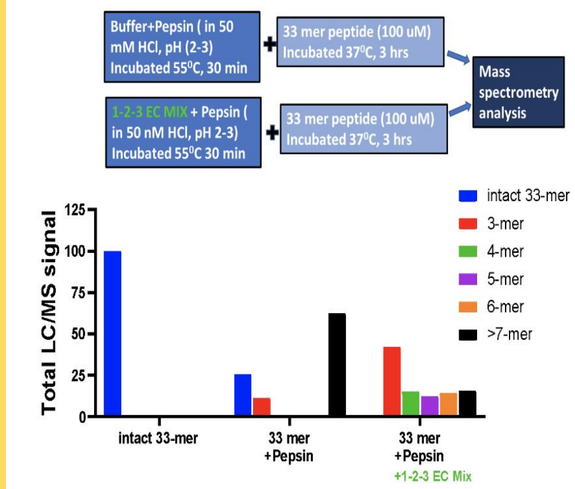


Figure 4: 1-2-3 EC MIX is effective in degrading the Immunogenic 33-mer peptide in the stomach acidic pH environment. Top). Protocol followed. **Bottom).**The LC/MS normalized peak signal corresponding to the intact 33mer and degraded into smaller products are plotted. Pepsin (in HCl) alone was not enough to break down 33-mer completely as evident from the presence of intact 33-mer gliadin peaks (blue bar) and residual larger mer's (>7, black bar).

Photo Credit: Aditi Avinash

Figure 5

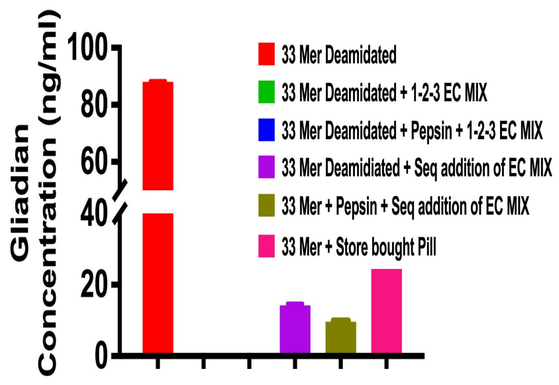


Figure 5: 1-2-3 EC MIX significantly degraded gliadin at pH=6 and at acidic pH 2-3 compared to sequential addition of 1/2/3 enzymes and **1-2-3 EC MIX** functions better than store bought pill as measured by Elisa G12 assay.

Photo Credit: Aditi Avinash

Results 4- Gliadin immunogenic epitopes treated with 1-2-3 EC MIX loses its ability in stimulating T-cell response

Figure 6

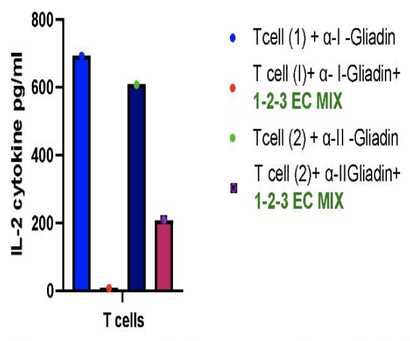


Figure 6: **Both immunogenic gliadin epitopes (α-I and II) when incubated with 1-2-3 EC MIX lost their immunogenicity in triggering T-cell response.** T cell (1) and (2) (details given in methods) are reactive to α-I and II gliadins respectively and this stimulation was reduced by the presence of **1-2-3 EC MIX** as measured by the reduction in the mouse IL-2 cytokine secretion.

Photo Credit: Aditi Avinash

Discussion

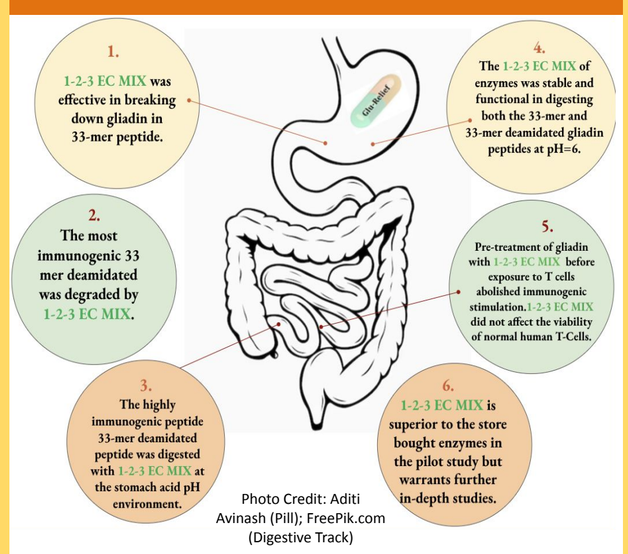
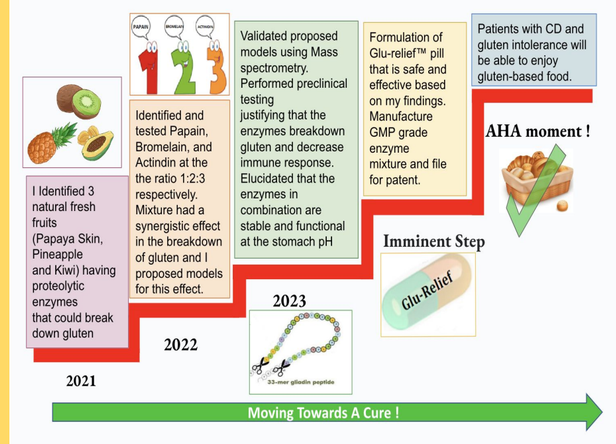


Photo Credit: Aditi Avinash (Pill); FreePik.com (Digestive Track)

Summary of Work



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Future Work

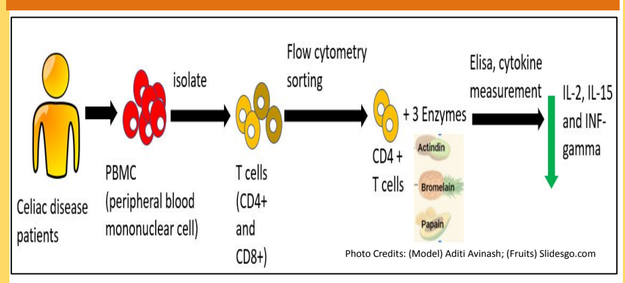

Photo Credits: (Model) Aditi Avinash; (Fruits) Slidesgo.com

Testing the efficacy of **1-2-3 EC MIX** in targeting and digesting gluten using human gluten-reactive intestinal T cell line (iTCL). These cells are cultured from the intestinal tissue collected from celiac disease patient.

Though I was planning on using the human reactive T cells this year, to my knowledge it was neither commercially available nor any lab in the USA have access to it. However there is a lab internationally that uses the iTCL cells. I have made contact with this lab, so I hope to collaborate with them in the future. This year I started working with physicians at children's hospital at colorado who treat celiac disease patients to understand the drug dosing strategies.

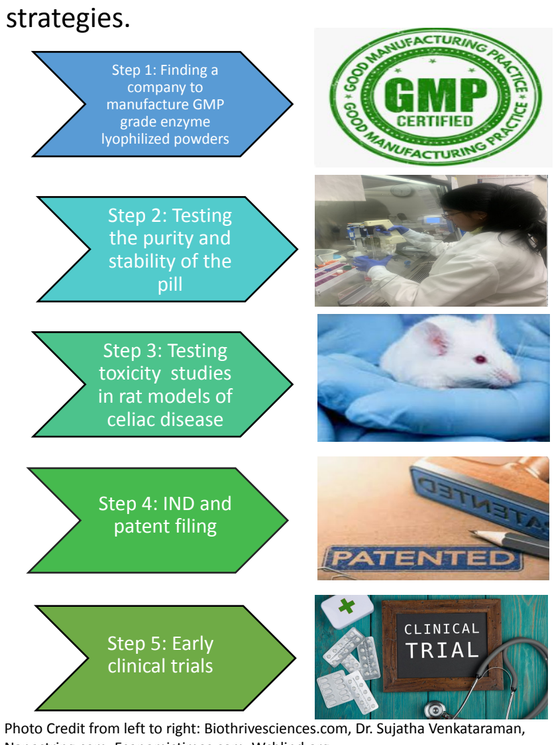

Photo Credit from left to right: Biothrivesciences.com, Dr. Sujatha Venkataraman, Nanostring.com, Economictimes.com, Wcblind.org

References

1. Jan Petersen, Jeroen van Bergen, Khai Lee Loh, Yvonne Kooy-Winkelaar, Dennis X. Beringer, Allan Thompson, Sjoerd F. Bakker, Chris J. J. Mulder, Kristin Ladell, James E. McLaren, David A. Price, Jamie Rossjohn, Hugh H. Reid, Frits Koning; Determinants of Gliadin-Specific T Cell Selection in Celiac Disease. *J Immunol* 15 June 2015; 194 (12): 6112–6122. https://doi.org/10.4049/jimmunol.1500161
2. Alves TO, D'Almeida CTS, Scherf KA, Ferreira MSL. Modern Approaches in the Identification and Quantification of Immunogenic Peptides in Cereals by LC-MS/MS. Front Plant Sci. 2019 Nov 14;10:1470. doi: 10.3389/fpls.2019.01470. PMID: 31798614; PMCID: PMC6868032.092. PMID: 28327674; PMCID: PMC5361186.
3. Qiao SW, Bergseng E, Molberg Ø, Xia J, Fleckenstein B, Khosla C, Sollid LM. Antigen presentation to celiac lesion-derived T cells of a 33-mer gliadin peptide naturally formed by gastrointestinal digestion. J Immunol. 2004 Aug 1;173(3):1757-62. doi: 10.4049/jimmunol.173.3.1757. PMID: 15265905.
4. Green PH, Fleischauer AT, Bhagat G, Goyal R, Jabri B, Neugut AI. Risk of malignancy in patients with celiac disease. Am J Med. 2003 Aug 15;115(3):191-5. doi: 10.1016/s0002-9343(03)00302-4. PMID: 12935825.
5. Podkaminer KK, Shao X, Hogsett DA, Lynd LR. Enzyme inactivation by ethanol and development of a kinetic model for thermophilic simultaneous saccharification and fermentation at 50 °C with Thermoanaerobacterium saccharolyticum ALK2. Biotechnol Bioeng. 2011 Jun;108(6):1268-78