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CardioRenal Therapeutics

Non-Confidential Data Synopsis

Vastiras®

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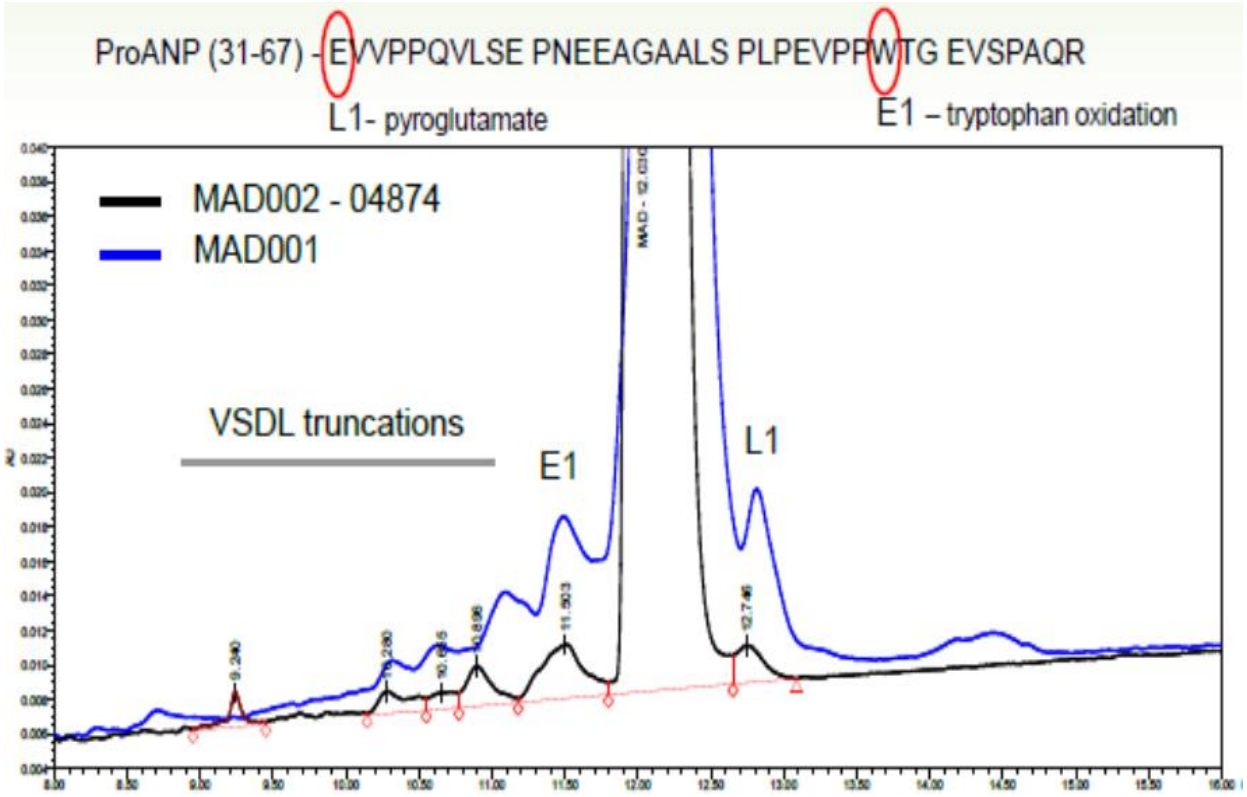
Recombinant Human MP3167 (rhMP3167)

- Recombinant API with greater purity profile than synthetic
- Recombinant vs. synthetic: commercial scale achievable; cost effective
- Madeleine owns the proprietary production technology



Recombinant Human MP3167
(rhMP3167)

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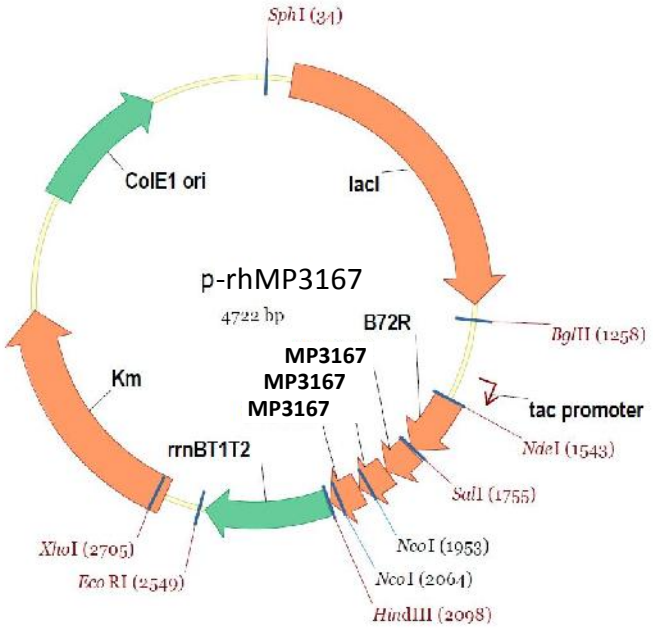
proANP 31-67
EVVPPQVLSEPNEEAGAALSPLPEVPPWTGEVSPAQR

B72R-MAD002 *(1mer)*
MKEVKSLLLDLQLLLEKVKNPENLKLSRMHTFDFYVPKVNATELKHLKALLEELKLLEEVLNLAPSKNLNVD**R**EVVPPQ
VLSEPNEEAGAALSPLPEVPPWTGEVSPAQ**R**

B72R-MAD002 *(3mer)*
MKEVKSLLLDLQLLLEKVKNPENLKLSRMHTFDFYVPKVNATELKHLKALLEELKLLEEVLNLAPSKNLNVD**R**EVVPPQ
VLSEPNEEAGAALSPLPEVPPWTGEVSPAQ**R**EVVPPQVLSEPNEEAGAALSPLPEVPPWTGEVSPAQ**R**EVVPPQVLS
EPNEEAGAALSPLPEVPPWTGEVSPAQ**R**



proANP 31-67 peptides were expressed as 1-mer and 3-mer fusions with B72R.
Blue sequence represents the fusion partner (B72R) and black sequences represent proANP 31-67 (“MADE002”). Trypsin cleavage sites are indicated by red arginine (**R**).

Fusion protein gene “concatemers” for the 3-mer were constructed for bacterial transformation.

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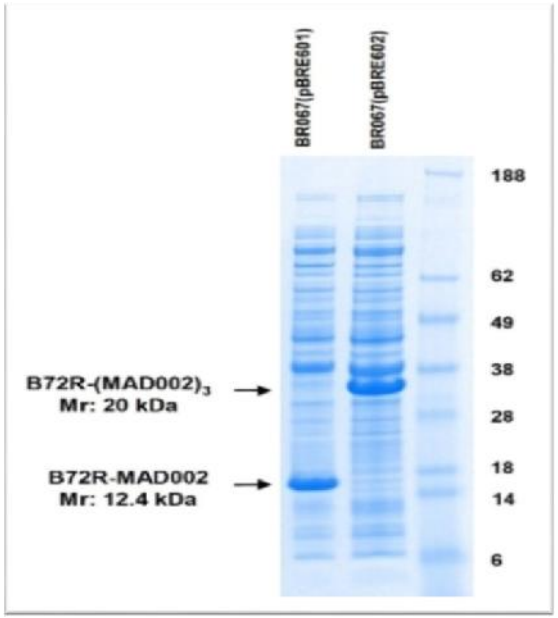
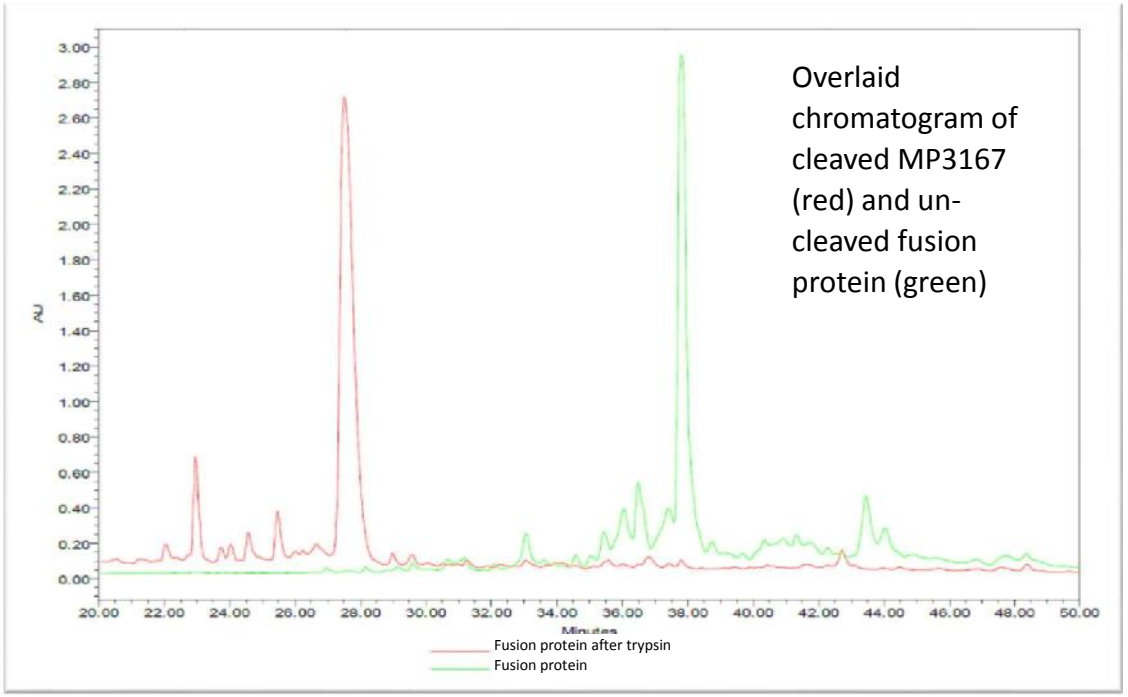


SDS-PAGE analysis of final culture samples demonstrate acceptable levels of expression of both 1-mer and 3-mer constructs under scaled conditions



Fusion protein digestion by single step trypsin treatment yields intact rhMP3167 (“MADE002”) by RP-HPLC under the conditions tested.

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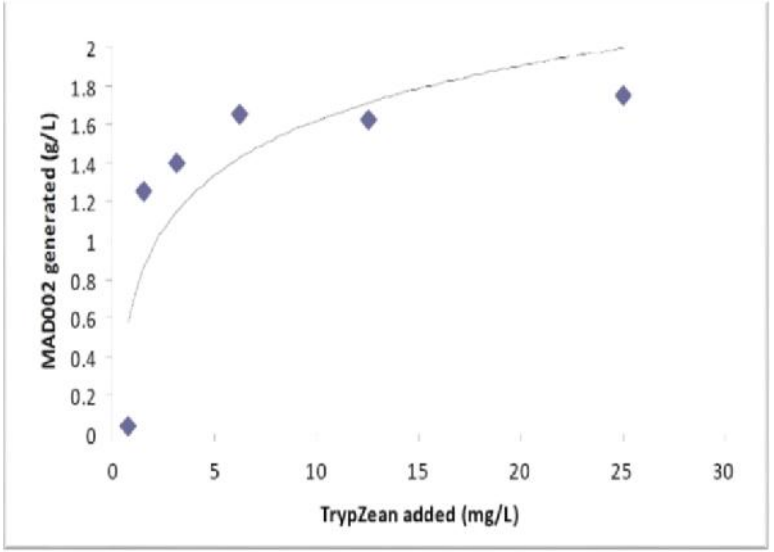
Original Process (TR547)

Harvested cells
↓
Homogenisation
↓
Centrifugation
(Clarification)
↓
Whole cell lysate
↓
pH Adjustment-1
(pH adjusted to 10.0 and then to 8.0)
↓
TrypZean® (Sigma-Aldrich) Digestion
(25 µg/ml Fermentate)
↓
pH Adjustment-2
(pH adjusted to 4.0)
↓
Depth and Bioburden-Reduction Filtration
↓
Reversed-Phase Chromatography
(Amberchrom CG300C)
↓
Anion-Exchange Chromatography-1
(ChromaSorb™ 50 mL device)
(Elute with 0.2 M NaCl)
↓
Anion-Exchange Chromatography-2
(ChromaSorb™ 50 mL device)
(Elute with 10 mM HCl)
↓
Final pH adjustment and Bioburden-Reduction Filtration
↓
Storage in Sterile Bags

Revised Process

Harvested cells
↓
Acetic acid extraction
(25% acetic acid, 2 extractions)
↓
TFF
(concentration and buffer exchange)
↓
pH Adjustment-1
(pH adjusted to 10.0 and then to 8.0)
↓
Trypsin (Roche) Digestion
(6.25 µg/mL Fermentate)
↓
pH Adjustment-2
(pH adjusted to 4.0)
↓
Depth and Bioburden-Reduction Filtration
↓
Reversed-Phase Chromatography
(Amberchrom CG300C,
(Elute with 15% IPA)
↓
Anion-Exchange Chromatography
(ChromaSorb™ 50 mL device)
(Elute with 0.2 M NaCl)
↓
Final pH adjustment and Bioburden-Reduction Filtration
↓
Storage in Sterile Bags



An enhanced downstream purification process was developed employing a single enzymatic release of rhMP3167 peptides followed by two column purification steps.

This initial process yielded ~1.5 gm API/L ferment.

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Assay Methodology	Quality Standard
Identification (HPLC)	Retention time of principal peak is within 0.3 min of reference
Related Impurities based on HPLC AUC	Total related impurity not more than 4.0%
Determination of whole mass identity and characterization of peptide impurities – LC/MS	Observed API mass is 3878.3 ± 2.0 Da (0.05%)
Related Substance of Higher Molecular Weight (dimers) based on elution profile	Sum of higher molecular weight related impurities not more than 0.6%
Bacterial Endotoxin using kinetic quantitative chromogenic method with Chromo-LAL	NMT 10 EU/mg
Host Cell Protein using Cygnus Technologies' enzyme-linked immunoabsorbant assay kit	NMT 50 ng/mg
Second Host Cell Protein Assay (to be determined by applicant as per FDA)	NMT 10 ppm
Host Cell DNA using Threshold Total DNA Assay kit	NMT 500 pg/mg
Bioequivalence (Potency/Efficacy) [to be developed in CMC Task #1]	90-110% of well characterized reference assay standard of mechanistic/clinical relevance
Osmolality	280 ± 15
pH	5.4 ± 2
Appropriate in-process testing or release testing to verify that no residual 73-amino acid fusion peptides(s) are present in the drug substance	Cleavage and purity criteria as described above

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