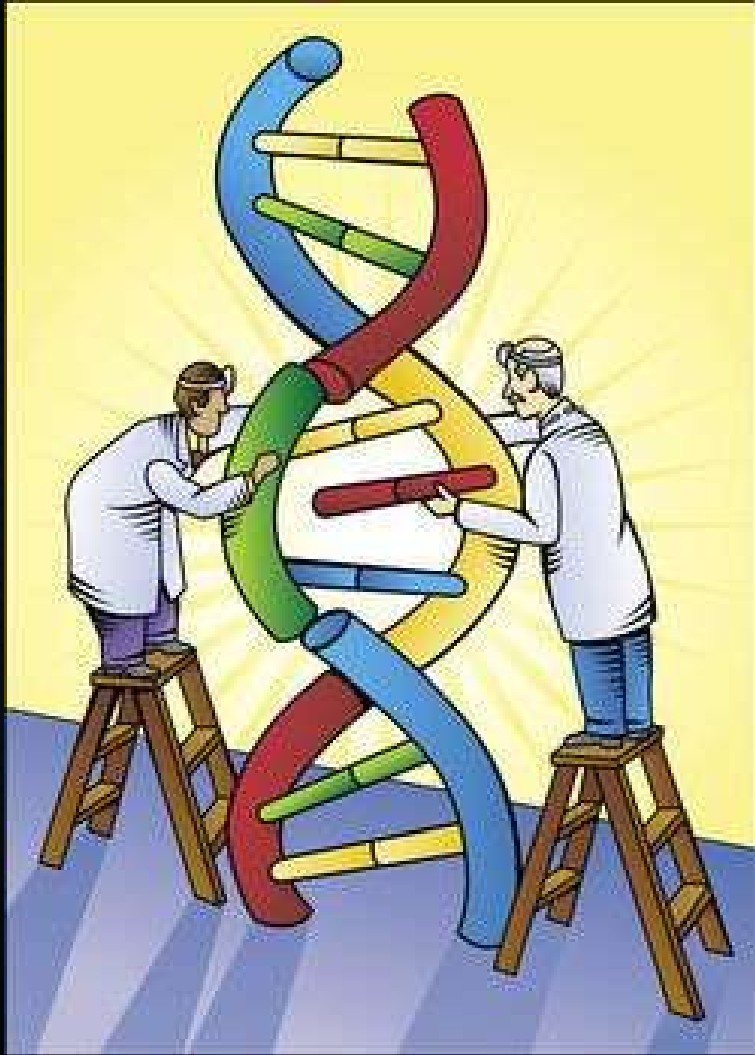


Gene Therapy

Bob Igo



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The Problem

- Biology
 - Some genes suck
 - Genes encode proteins*
 - Some of the proteins cause disease

* They encode RNA, too, but let's think about proteins.



An Analogous Problem



- Software
 - Some code has bugs
 - Compiling code produces binaries
 - Parts of the binaries cause bugs

The Solution

- Biology
 - Remove the bad genes
 - Replace with good genes



- Software
 - Eliminate the buggy code
 - Replace with good code



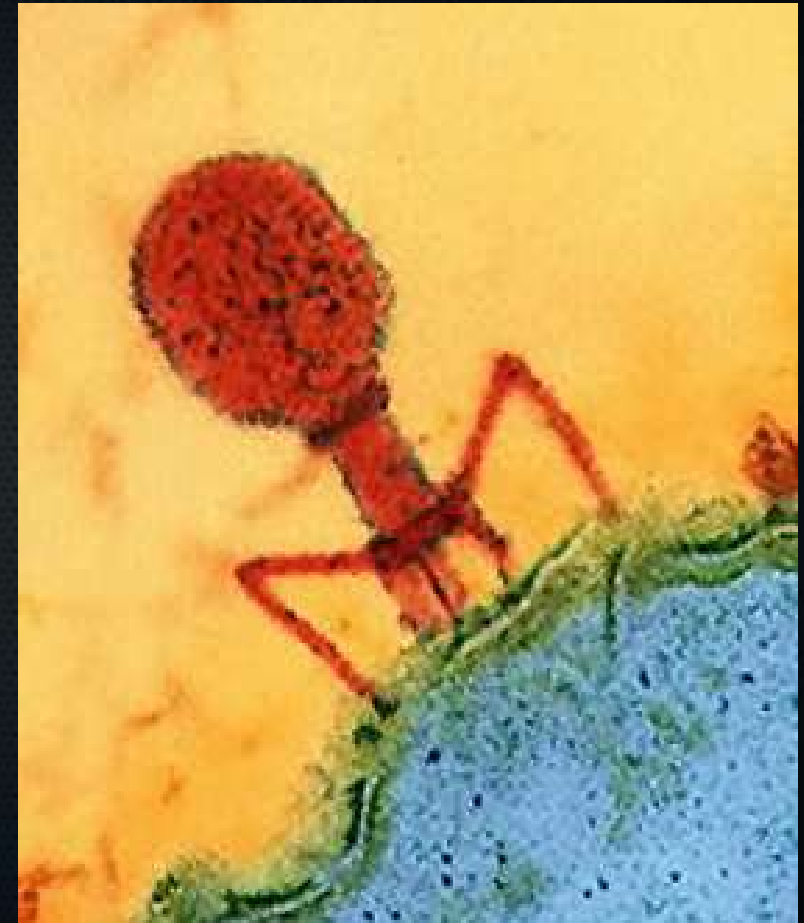
Toolset

- Gene therapy relies on discovered tools from nature
 - viruses
 - repurposed
 - limitations



Viruses, Nature's Code-injection Exploit

- All viruses inject DNA into target cells
 - retroviruses inject it in random places
 - This can cause cancer
- Different viruses target different organs
- Target cells reproduce this DNA as a side-effect of normal operations
- Self-replicating viruses inject their full genome
 - The cells make more virus
 - Eventually the cells explode, and the virus spreads
- All of this makes your immune system react
 - Survivors produce antibodies against the virus

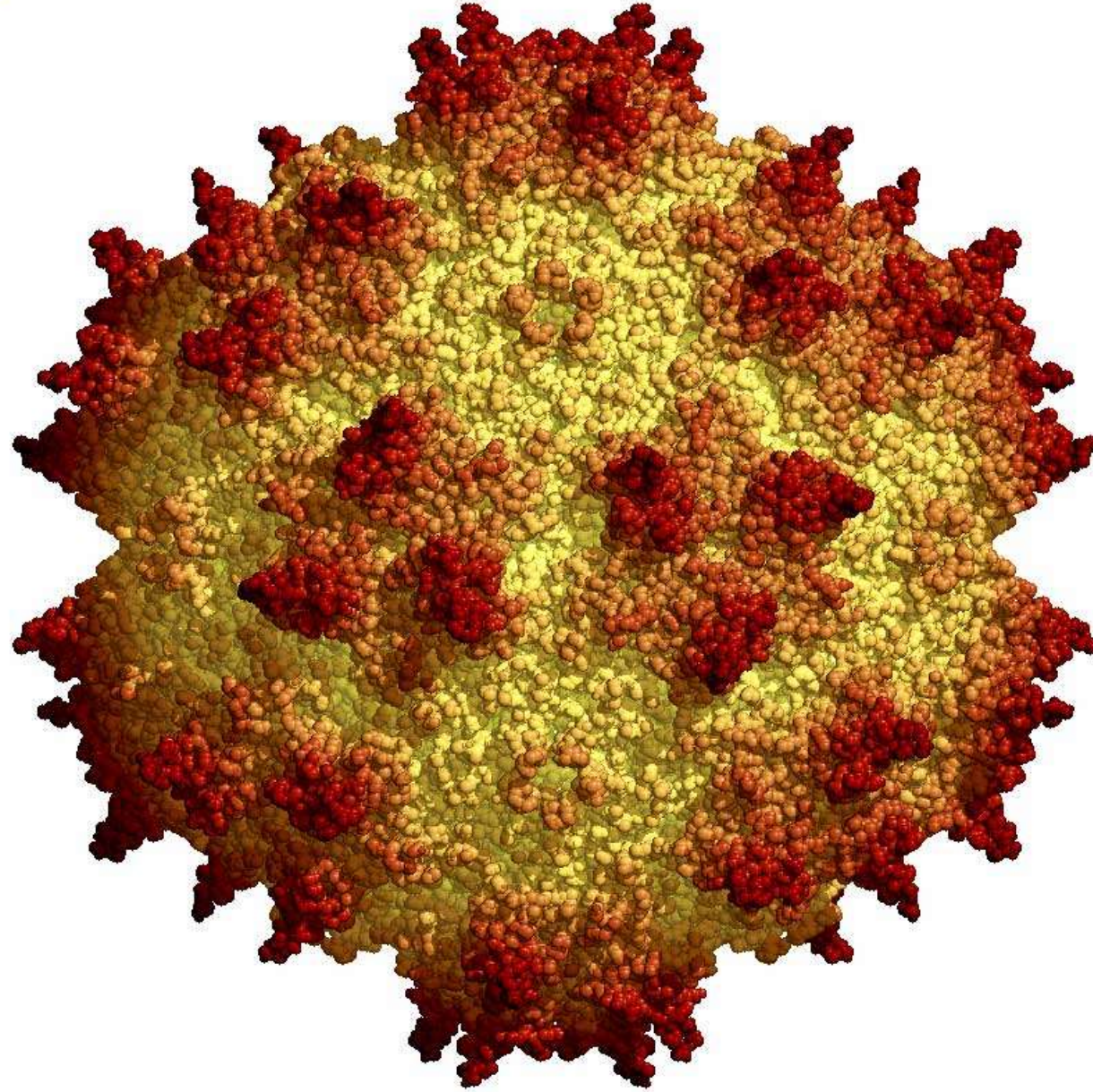


Adeno-Associated Virus (AAV)

- Benefits over other virus types:
 - Injects DNA in a specific, safe part of the human genome
 - Does not self-replicate
 - Does not generate much immune response

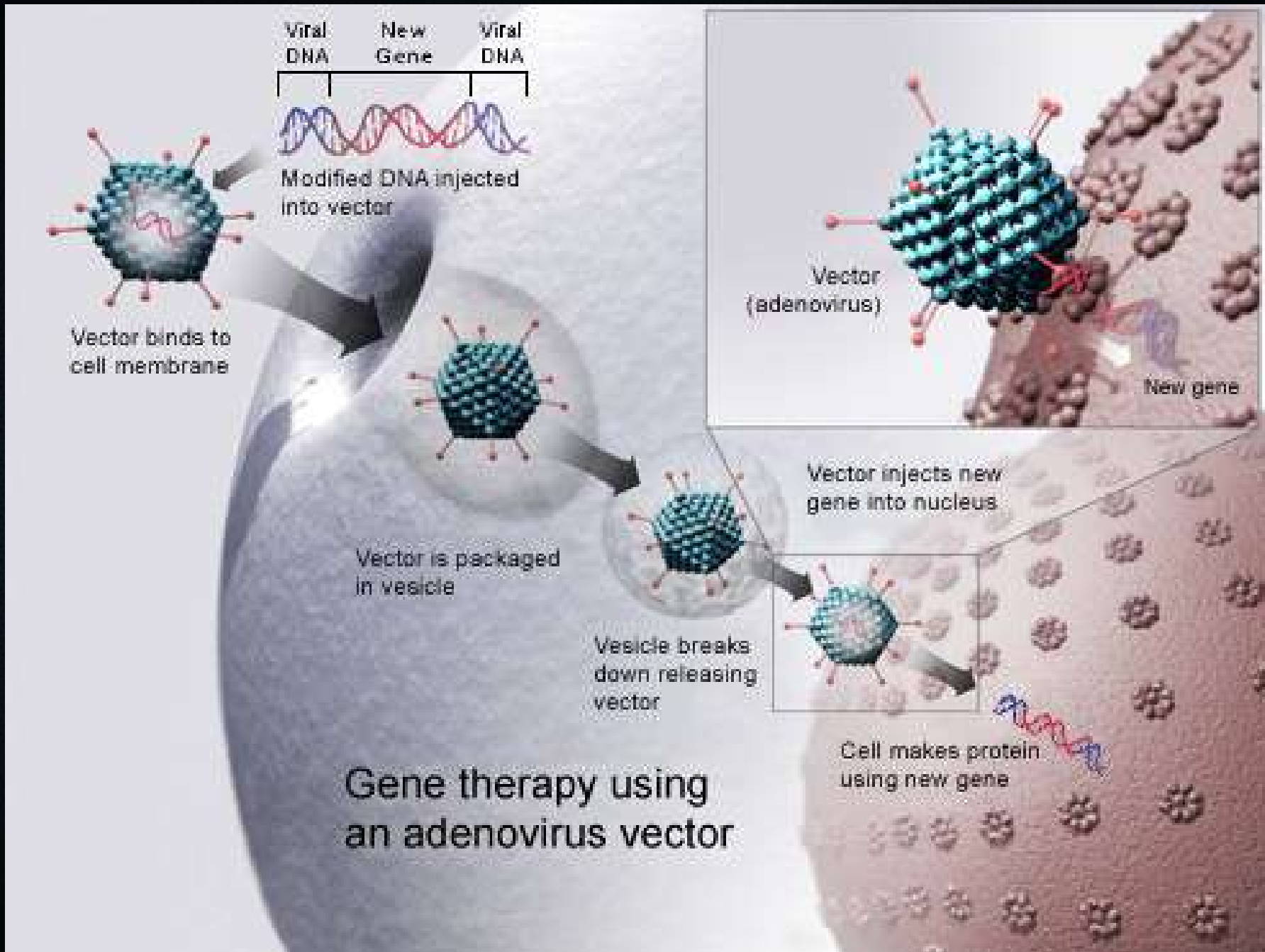
20Å

Parvoviridae: Adeno-associated virus PDB_ID: 1LP3



AAV

- Drawbacks
 - Unmodified virus has ~5000-base genome
 - When altered for gene therapy, "payload" gene can't exceed that size
 - Ends up in the liver
 - Good if you're targeting the liver
 - Bad if you're not targeting the liver
 - AAV occurs in nature
 - Patients may already have been infected and have antibodies



Example: Hemophilia B

- Single-gene disorder (F9)
- Small gene size (1386)
- Gene is in the liver
- The bad gene is causing no harm but not causing a needed good
- Adding the good gene would cure the disorder
 - Removing the defective gene is unneeded

```
• 1   ATGCAGCGCG TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCTTTTA
• 61  GGATATCTAC TCAGTGCTGA ATGTACAGTT TTTCTTGATC ATGAAAACGC CAACAAAATT
• 121 CTGAATCGGC CAAAGAGGTA TAATTCAGGT AAATTGGAAG AGTTTGTTCAGGGAACCTT
• 181 GAGAGAGAAT GTATGGAAGA AAAGTGTAGT TTTGAAGAAG CACGAGAAGT TTTTGAAAAC
• 241 ACTGAAAGAA CAACTGAATT TTGGAAGCAG TATGTTGATG GAGATCAGTG TGAGTCCAAT
• 301  CCATGTTTAA ATGGCGGCAG TTGCAAGGAT GACATTAATT CCTATGAATG TTGGTGTCCC
• 361  TTTGGATTTG AAGGAAAGAA CTGTGAATTA GATGTAACAT GTAACATTAA GAATGGCAGA
• 421  TGCGAGCAGT TTTGTAAAAA TAGTGCTGAT AACCAAGGTGG TTTGCTCCTG TACTGAGGGA
• 481  TATCGACTTG CAGAAAACCA GAAGTCCTGT GAACCAGCAG TGCCATTTC ATGTGGAAGA
• 541  GTTTCTGTTT CACAACTTC TAAGCTCACC CGTGCTGAGG CTGTTTTTCC TGATGTGGAC
• 601  TATGTAAATT CTACTIONAAGC TGAACCATT TTGGATAACA TCACTCAAAG CACCCAATCA
• 661  TTTAATGACT TCACTCGGGT TGTGGTGGA GAAGATGCCA AACCAGGTCA ATTCCCTTGG
• 721  CAGGTTGTTT TGAATGGTAA AGTTGATGCA TTCTGTGGAG GCTCTATCGT TAATGAAAAA
• 781  TGGATTGTAA CTGCTGCCCA CTGTGTTGAA ACTGGTGTTA AAATTACAGT TGTCGCAGGT
• 841  GAACATAATA TTGAGGAGAC AGAACATACA GAGCAAAGC GAAATGTGAT TCGAATTATT
• 901  CCTCACCACA ACTACAATGC AGCTATTAAT AAGTACAACC ATGACATTGC CCTTCTGGAA
• 961  CTGGACGAAC CCTTAGTGCT AAACAGCTAC GTTACACCTA TTTGCATTGC TGACAAGGAA
• 1021 TACACGAACA TCTTCCTCAA ATTTGGATCT GGCTATGTAA GTGGCTGGGG AAGAGTCTTC
• 1081 CACAAAGGGA GATCAGCTTT AGTTCTTCAG TACCTTAGAG TTCCACTTGT TGACCGAGCC
• 1141 ACATGTCTTC GATCTACAAA GTTCACCATC TATAACAACA TGTTCTGTGC TGGCTTCCAT
• 1201 GAAGGAGGTA GAGATTCATG TCAAGGAGAT AGTGGGGGAC CCCATGTTAC
      TGAAGTGGAA
• 1261 GGGACCAGTT TCTTAACTGG AATTATTAGC TGGGGTGAAG AGTGTGCAAT GAAAGGCAAA
• 1321 TATGGAATAT ATACCAAGGT ATCCCGGTAT GTCAACTGGA TTAAGGAAAA AACAAAGCTC
• 1381 ACTTAA
```

2013 Clinical Trials

- No negative outcomes
- F9 expression rose for all participants
 - 80% no longer need prophylaxis



Example: Alpha-1 Antitrypsin Deficiency

- Single-gene disorder (Serpina1)
- Small gene size (1257)
- The bad gene is causing harm as well as denying a needed good
- Adding the good gene would cure half the disorder
 - Removing the defective gene is ideal

```
• 1   ATGCCGTCTT CTGTCTCGTG GGGCATCCTC CTGCTGGCAG GCCTGTGCTG CCTGGTCCCT
• 61  GTCTCCCTGG CTGAGGATCC CCAGGGAGAT GCTGCCCAGA AGACAGATAC ATCCCACCAT
• 121 GATCAGGATC ACCCAACCTT CAACAAGATC ACCCCCAACC TGGTGTGAGTT CGCCTTCAGC
• 181 CTATACCGCC AGCTGGCACA CCAGTCCAAC AGCACCAATA TCTTCTTCTC CCCAGTGAGC
• 241 ATCGCTACAG CCTTTGCAAT GCTCTCCCTG GGGACCAAGG CTGACACTCA CGATGAAATC
• 301 CTGGAGGGCC TGAATTTCAA CCTCACGGAG ATTCCGGAGG CTCAGATCCA TGAAGGCTTC
• 361 CAGGAActCC TCCGTACCCT CAACCAGCCA GACAGCCAGC TCCAGCTGAC CACCGGCAAT
• 421 GGCCTGTTCC TCAGCGAGGG CCTGAAGCTA GTGGATAAGT TTTTGGAGGA TGTTAAAAAG
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• 601 GACAGAGACA CAGTTTTTGC TCTGGTGAAT TACATCTTCT TTAAGGCAAATGGGAGAGA
• 661 CCCTTTGAAG TCAAGGACAC CGAGGAAGAG GACTTCCACG TGGACCAGGC GACCACCGTG
• 721 AAGGTGCCTA TGATGAAGCG TTTAGGCATG TTTAACATCC AGCACTGTAA GAAGCTGTCC
• 781 AGCTGGGTGC TGCTGATGAA ATACCTGGGC AATGCCACCG CCATCTTCTT CCTGCCTGAT
• 841 GAGGGGAAAC TACAGCACCT GAAAATGAA CTCACCCACG ATATCATCAC CAAGTTCCTG
• 901 GAAAATGAAG ACAGAAGGTC TGCCAGCTTA CATTACCCA AACTGTCCAT TACTGGAACC
• 961 TATGATCTGA AGAGCGTCCT GGTCAACTG GGCATCACTAAGGTCTTCAG CAATGGGGCT
• 1021 GACCTCTCCG GGGTCACAGA GGAGGCACCC CTGAAGCTCT CCAAGGCCGT GCATAAGGCT
• 1081 GTGCTGACCA TCGACGAGAA AGGGACTGAA GCTGCTGGGG CCATGTTTTT AGAGGCCATA
• 1141 CCCATGTCTA TCCCCCCCCG GGTCAAGTTC AACAAACCCT TTGTCTTCTT AATGATTGAA
• 1201 CAAAATACCA AGTCTCCCCT CTTTCATGGGA AAAGTGGTGA ATCCCACCCA AAAATAA
```

2011 Clinical Trials

- No negative outcomes
- No "therapeutic" levels of A1AT resulted in any participant
 - Best result was 1/200th of target



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- SerpinA1 DNA
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