What is 'gene therapy'? Using examples, describe how gene therapy can be achieved, when it is appropriate, and outline some of the key safety considerations.

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Gene therapy is using genetic material (DNA/RNA) to treat disease. There are numerous methods to achieve gene therapy that can be temporary (in the case of somatic cell gene therapy) or permanent (in the case of germline gene therapy), this distinction comes with its own safety and ethical concerns. The methods for actually getting the therapeutic genetic material into cells whether they be viral or non-viral, also come with their own safety concerns. But the actual editing technology of CRISPR and its subsequent adaptation to make it safer has increased its viability and with it brought more investment and research making gene therapy an exploding area. It can be done on individuals with a disease, or during the first stages of development (at a one or 2 cell stage) fundamentally changing our genetic code. The scientific capability appears to be getting ahead of the ethics (and possibly legal systems) which in itself brings safety concerns, alongside the debates about what conditions should these techniques be used on? This short essay will not have time to examine this topic fully but will explore a few methods of how gene therapy can be performed, with comments on safety considerations, and make comments on which diseases and circumstances it could be appropriate.

To actually get the DNA into the host cell this can be achieved through viral or non-viral methods. In the case of viral the DNA is packaged into a virus wither it be adenoviruses, adneo-associated viruses or lentiviruses so depending on your goal you can use anyone of these. Adenoviruses (AAVs) can put DNA in a non-permanent manner as the viruses are eventually eliminated by the innate immune system and can package up to 7.5kbps. Adeno-associated viruses are smaller but are non-pathogenic. AAVs integrate into a site on chromosome 19 but this function can be altered out and has been used against haemophilia, lipoprotein lipase deficiency, and retinal dystrophy (by AAV delivering RPE specific protein cDNA into the subretinal space). Lentiviruses are retroviruses with an RNA genome which gets reverse transcribed to DNA and can be transcribed, most of these are HIV derivatives engineered to be safe. So, it can integrate DNA long term and has been used to treat things like x-linked adrenoleukodystrophy. Retroviral therapy has also been used to treat successfully treat SCID by transfecting healthy adenosine deaminase cDNA into patient T-cells before putting them back into patients, these transduced lymphocytes survived long term. But viral based treatment of SCID has also cause failure of B and T cell function and caused cancer and death through of site effects as it integrated close to an oncogene causing upregulation. Also, Jesse Gelsinger, must be mentioned when considering safety considerations with regards to gene therapy and viruses as he unnecessarily died from it. He had a mild/mosaic form of ornithine transcarbamylase deficiency, which affects urea processing and causes ammonia accumulation and in severe cases is fatal at birth, but his milder form just meant a manageable restricted diet and medication. In his case he had an immune reaction to the virus, which lead to multiple fatal organ failure and brain damage, and it turned out that lead investigators had overlooked red-flag symptoms in animal models because they had financial interest in the company that aimed to make profits from this research. Also, viruses have the risk of crossing over in the sense that if someone had normal and untreated HIV/coronavirus/flu then had gene therapy via a viral vector and these viruses interacted and adapted it could become a contagious genome editing virus that unintendedly changes human genomes globally.

Arguably safer vectors for gene therapy like liposomes can be used to deliver DNA like a "trojan horse" with hydrophobic and hydrophilic residues that bound to DNA can it across cell membranes. This method was used with limited degrees of success in 2015 to safely treat 78 cystic fibrosis patients. Nanoparticles can also be used in the form of synthetic polymers which form nanoparticles when

mixed with DNA that can be endocytosed thus delivering gene therapy. Other methods on non-viral like injection of naked DNA, gene guns, magnetofection, sonoporation and electroporation can be used but could have unseen implication so it would be important to extensively test and perfect methods.

Gene therapy can use short interfering or short hair pin RNA to cause mRNA degradation by binding and direction of RNA induced silencing complex to cleave the target mRNA. SiRNA is short (22bp) and double stranded RNA of the gene of interest, which I then digested by DICER and guides RISC to the target, if the RNA is too large it can cause an antiviral response, but both Si and Sh RNA have been used in Huntington's treatment. Facioscapulohumeral muscular dystrophy was reversed by targeting FRGI with shRNA (delivered by AAVs) in FGRI overexpressing mice. Gene therapy can also use CRSIPR/Cas9 DNA "cut and paste" technology, which has been modified to reduce unwanted off target effects. This could be used on the over 32,000 SNPS identified as pathogenic. With this we have unprecedented capability to fundamentally treat/eliminate genetic diseases, this calls into question which diseases, I would argue with all the risks associated with it we should be focused on treating only the most severe otherwise untreatable diseases through theses methods, as diseases that can be managed through other means does not warrant the risk. I would also argue that changing the human genome during development not only is open to unexpected risks, but a slippery slope as to which diseases you eliminate, as it could start with severe and fatal SCID, then lead to the elimination of the manageable form that Gelsinger had, to then achondroplasia, to eye colour and so on. CRISPR babies have already been attempted with He Jiankui attempting to make HIV resistant children was met with staunch debate around what could arguable be an important cause, but I believe the reaction towards it was not positive. And if there are huge reservations about changing mice to make them resistant to and eliminate tick borne diseases (in America) due to the ecological impact, I cannot imagine we are anywhere near making germline changes in humans even if it is just for resistance.

The safety concerns around gene therapy and their potential negative effects through unseen immune responses (or unexpected gene interactions or unintended viral spread) but also safety considerations about those developing or giving the treatment. Gelsinger showed that mislead financial incentives, just like mislead ideology in WW2 allowed doctors to do unnecessary and fatal experiments in the name of progress. I am not saying that this was equivalent to the atrocities of Nazi doctors, but I would argue that taking a do no harm oath is not enough to stop misguided experimentation with gene therapy. Currently a major safety consideration is that we lack a good enough legal and monitoring framework because currently depending on where you live this technology can be bought by anyone of the internet. And should someone want to permanently change germline cells they can and ultimately play god, they can, and it is not only limited to researchers who took the Hippocratic oath and those who didn't it is anyone. The advocates in America argue for this to be free to use within the "biohacker" community as they believe that if everyone has it no single group can gain the upper hand with "super humans" is misguided and dangerous for the reasons above, and I would argue giving everyone access to it would only increase the risks of future problems/atrocities.