Daisy Q&As

Q: Why is this significant?
This research could have longer term benefits to understanding and combatting allergies to dairy products. The AgResearch team aimed to produce milk which contained less of beta-lactoglobulin (BLG) milk protein not present in human milk which is allergenic. Two to three percent of infants are allergic to cow's milk, and BLG allergies make up a large part of that percentage.

Q: Why did you start this project with mice?
The micro RNAs (Ribonucleic acids) that that were designed for reducing the milk protein beta lactoglobulin were first tested in cell culture, a relatively easy and fast test. While this test gives a good indication that certain micro RNAs are functional, there's no guarantee that these micro RNAs will also working animals. Since it's very expensive to produce genetically modified cows, we decided to first test our micro RNAs in a cheaper mouse model.

Q: At what point you take the research to a calf - what made you decide this was feasible?
Having shown that the micro RNAs worked in the mouse model, we were comfortable to move ahead and use them in cows.

Q: What does it mean to "knock down" a gene?
Differing from a gene "knock out", which entails the functional disruption of the gene, we used a method termed RNA interference. RNA interference prevents messenger RNA from being converted into protein. In contrast to "knock outs", this method does not completely block protein production and is coined "knock down" to indicate that there are still residual amounts of proteinproduced. But in the case of the cow, we could no longer detect BLG in her milk.

Q:What are the implications for the future?
While the project was motivated by the prospect of generating cows that produce hypoallergenic milk, we first of all consider this research a model, thatwill allow us to testwhether the milk is indeed less allergenic than normal cow's milk.

Q: What kind of allergies would this address?
It does exclusively address allergies that are due to an immunogenic response against BLG. Some people can be allergic to any bovine milk proteins but BLG, probably due to its absence in human milk, is a major cow's milk allergen.

Q: How does this relate to lactose intolerance?
It's not related. Lactose intolerance relates to the lack of an enzyme that breaks down the milk sugar lactose.

Q: Does it seem feasible to "engineer" dairy cattle so as to produce this type of milk? Or is it prohibitively expensive?
It is feasible to 'engineer' dairy cattle with that kind of milk (we're in the process of producing more animals) but it is indeed expensive and will, at least initially, be limited to few animals.

Q: What is the next research step?
We want to breed from Daisy and determine the milk composition and yield from a natural lactation. We will also perform cell culture tests to determine whether the BLG-free milk has hypoallergenic properties.

Q: You attribute the missing tail to a mutation entirely separate from the gene-transferring process?
We are in the process of confirming that the missing tail is linked to an epigenetic defect, rather than to the gene transferring process. This congenital abnormality is rare in cows and not something we have seen in animals we have cloned previously.

Q: Can you outline in simple language how miRNA works in this case?
Micro RNAs bind messenger RNAs sequence-specifically and prevent the messenger RNAs from being converted into protein. We designed micro RNAs that bind both cow and sheep messenger RNAs of BLG and tested whether and which of the chosen miRNAs would prevent protein production. Efficient micro RNAs are expected to greatly reduce production of the targeted protein while still allowing the production of residual protein amounts. In our mouse model, we did indeed detect residual protein, but, somewhat surprisingly, in the case of the cow, we could no longer detect BLG in her milk.

Q: How do you work up the miRNAs to screen in the mouse model?
The mouse model is more complicated than the cell culture sceening. Mice, as humans, do not produce BLG. Thus, in order to use mice as a model for miRNA reduction of BLG, one needs genetically modified mice that produce the protein. We obtained such mice from the Roslin Institute in Edinburgh and crossed them with genetically modified mice containing some of the best-working micro RNAs from the cell culture test. In a confirmation of our cell culture screening, we observed that double transgenic mice (BLG + micro RNAs) produced very little BLG in comparison with mice that do not contain micro RNAs (BLG only).

Q: How do you create the calf that carries these genetic modifications? Did she carry other modifications beyond the miRNAs?
We used the technique of “cloning” that was famously introduced with Dolly, the clone sheep. The genetic modification, DNA copies of micro RNAs, were inserted in cow cells in cell culture. These genetically modified cells were used as “donors” for “cloning”. “Cloning” starts with the removal of the maternal genetic material from an unfertilized egg. Following this, the removed maternal DNA gets replaced by a cell nucleus from the “donor” cell that contains the genetic modification. Embryo are then developed to blastocysts, spheres of about 100 cells, and transferred in foster cows.

Q: You refer to the milk being "hormonally induced" - is that an artificial way to produce milk before the animal is mature? Would that milk have different composition?
Yes, to avoid the delay of two years before a natural lactation, the milk we analysed was from an induced lactation which can be performed at a calf’s age of about seven months. There is compelling evidence by various research groups that the milk composition is very similar between milks from induced and natural lactations.

Q: Would a cow that carried these miRNAS produce consistent (and low BLG) milk all her life?
We indeed expect that the miRNAs persistently knock down BLG and hope to get confirmation for that from other and naturally-lactating cows.

Q: The other proteins (caseins) go up. Is that good?
We don't know yet. First of all, we will have to determine whether the lack of detectable levels of BLG will impact on milk yield during natural lactation. High casein milk may result in enhanced cheese yields. This could be trialled in a cheese making experiment using milk from a natural lactation.

Q: What are your thoughts on the implications of your work? Could this have applications any time soon?
The great interest in BLG undoubtedly derives from its role as a major milk allergen. Thus, we would like to investigate whether the BLG-free milk has hypoallergenic properties. We are currently using a cell culture model to answer this question. We first of all consider our genetically modified cow a great tool to study allergenicity and do not envision any practical application any time soon.

Q: What's the difference between transgenic animals and cloned animals?
Cloned animals are genetically identical, younger twins of the animal whose cells were used to replace the maternal genetic material from an unfertilised egg.
Transgenic animals carry additional pieces of DNA in their genome. There is a multitude of techniques to make transgenic animals and cloning is just one of them. Employing cloning to produce transgenic animals involves the use of genetically modified cells as DNA donors instead of unmodified cells.

Q: What's the potential benefit to New Zealand of this work?
This work increases the image of New Zealand in the field of science on the world stage given the importance of the research, but is otherwise is difficult to quantify at the moment, given the discovery nature of the study and also the fact we operate within one of the world's strictest GM regulatory environments.

Q: How do you know this milk is safe for human consumption?
We don't. This is very much at the discovery phase, and much more work needs to be done. Under NZ's current GM legislation, it is not able to be consumed.

Q: Then why are you publicising it now?
It's a significant scientific achievement. No scientists have managed to "knock down" BLG before, and there are still many more questions to be answered.

Q: How does an allergic reaction to BLG manifest in children?
Reactions can vary from hives or eczema to vomiting and diarrhoea.

Q: Will this discovery help adults who have milk allergies?
BLG allergies are most commonly found in infants who outgrow them by the time they turn 3.

Q: How long away is it before people can buy hypo-allergenic milk?

This work is still very much at the discovery stage. We have shown it is possible to suppress BLG, but there is much more research to be done, and even if it does prove possible to do this on a larger scale, with New Zealand's current legislation, it wouldn't be possible to produce this commercially.