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**UNIT 13**

**Genetically Modified Organisms**

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**Thomas E. Newberry**



*Mr. Newberry is the Vice President of Corporate Communications at GTC Biotherapeutics in Framingham, MA. He is responsible for GTC's corporate communication programs - including investor and public relations, media relations, presentations and publications.*

**What is your company about?**

GCT Biotherapeutics is about serving the therapeutic protein marketplace. We do that by developing proteins in transgenic animals so that we recover these therapeutic agents from their milk.

**What is the current focus at GCT Biotherapeutics?**

We have actually two types of proteins that we are actively pursuing. One is what's known as a monoclonal antibody, or are sometimes known as antiglobin fusion proteins. These are specialized proteins typically needed in very large volumes that are difficult for a traditional bioreactor to make because of the large volumes involved.

The second kind of protein that we got involved in is what's known as a difficult to express protein, one that just doesn't produce very well inside of a cell culture in a stainless steel tank.

**What is an example of your main protein of interest?**

Our lead program is something called Antithrombin III. Antithrombin is a protein made normally in people's blood, and it participates in the coagulation process. It has both anti-coagulant and anti-inflammatory properties.

It is on the market today out of human blood plasma. It is a protein that is very difficult for what's known as a recombinant system to make. We have been able to make that in the milk of transgenic goats. And what we've been doing is breeding these animals, milking them, and recovering the protein. It's in studies right now about to enter an efficacy study to treat the condition known as hereditary deficiency, for those people that don't make enough of Antithrombin in their own blood, and therefore are prone to blood clots.

**Why do you produce it in goats?**

There are two answers to that. One is we produce it in transgenic animals because you can't produce it in a traditional bioreactor. We have chosen goats for many of our programs because a goat is a relatively good milking animal. It's a good dairy animal. It's sociable and relatively easy to work with. It has a relatively short gestation period and early sexual maturity, so you can get to them relatively quickly. The goats actually have performed very well across a broad range of proteins in developing transgenic animals that successfully produce proteins in their milk.

**What are the major functions of mammary glands to produce proteins?**

We take advantage of the features of a mammary gland. It is a source of rich liquid proteins as well as fats and energy for the offspring of any species, whether it's human, goat, cow, or any mammalian species.

What we're doing here is using a bit of, if you want to think of it this way, DNA

programming to get the mammary glands simply to produce a protein of therapeutic interest to man, in addition to all the other proteins it might make, and then we take the resulting fluid and you don't really think of it as milk so much. Usually when you use the term "milk" people think of a food source. The milk is really a production media for the protein and we then take all the milk parts away from the milk to get to this therapeutic protein.

And what we're doing here, by taking advantage of the mammary gland's ability to make proteins, is what enables this technology to get to very large production volumes in relatively low-costs ways. And secondly, because of the mammary gland's ability to make such a broad array of proteins, we can actually make proteins that are difficult for other traditional "tank bank" systems to do.

#### **How do you make a protein?**

Once we make a decision about what protein we're interested in developing as a therapeutic product, we get the DNA for that basic protein. More often than not, it's actually a human protein so you're getting a little bit, if you will, of human DNA for that protein.

And then we make what we term a "construct" out of it. Now a construct simply puts other small pieces of DNA programming on to it. The primary piece of interest is what's known as the beta casein promoter. A beta casein promoter is what ensures that this protein, once it is produced in the animal is produced only in its milk and not somewhere else in the animal's body that might lead to something unknown in the animal's health.

#### **What are the protein promoters?**

The beta casein gene is the promoter gene. We also use some insulators and isolators so that we end up with, as I said, a construct. This DNA construct is what's introduced to the animal's embryo and as the embryo grows it incorporates this little bit of genetic programming such that when the goat is born, the goat's milk when it matures will have this protein in it, but the protein won't be active anywhere else in the goat's body.

#### **What do you then do with the construct protein?**

Actually much of the technology is similar to what's used in human fertility. What we do is we harvest eggs from donor moms. We introduce the genetic material into those eggs and then we re-implant the eggs into the recipient, and then the animal simply gestates normally, incorporating this transgene into its growing fetus such that the resulting animal that is born is transgenic. It has the gene for this desired protein within its genomic makeup.

And then at that point, we will actually test the animal's tissues to ensure that we have the desired genetic makeup of the animal, and then as the animal matures we then start testing the milk to ensure that it's producing the desired protein the way we want it to.

#### **What technique do you use to get the construct into an embryo?**

We've used two technologies. One is known as "microinjection" where you take this construct and you take an extremely fine needle and you physically insert it into the one-celled embryo, a fertilized egg. And you wait for that gene to be incorporated during development into the growing fetus. It's a fine technology and it's well established.

We've also turned to "nuclear transfer." Nuclear transfer is a technology we've started using here because it offers certain advantages in terms of knowing when we will get a desirable transgenic animal. Nuclear transfer is the basic science behind cloning. What we're doing is we're taking this genetic construct that I've told you about we're putting into cell lines and growing it up in cell

lines. We've enucleated the egg from the donor mom and put the cell line material into the egg.

As that egg develops into a fetus and grows into an animal, we know that the animal being born will be transgenic, and therefore it offers a more deterministic way of knowing when will we achieve a transgenic animal than the microinjection method offers, and thereby we're able to be more schedule-predictive with the technology.

#### **Why isn't microinjection as precise as nuclear transfer?**

With microinjection, you're depending upon genetic incorporation during cell division. Sometimes that happens, sometimes it doesn't; sometimes it happens very late in the cell division process. And you don't know what type of animal you're going to get, a non-transgenic, a transgenic, or a partly transgenic animal. You don't know that until the animal is born.

Whereas with nuclear transfer, because that material is already incorporated in the cell line and you know that genetic profile, you know the genetic profile of the animal before the animal is born.

#### **What is the difference between a transgenic and a cloned animal?**

A transgenic animal simply has a transgene of interest within its genomic makeup. A cloned animal is developed through nuclear transfer, and typically has a common genetic profile with another animals.

#### **How long does it take for a goat to produce milk?**

Well, it takes them about seven months to get to sexual maturity, at which point you can get them pregnant again. Now you might get some early lactation off of an animal, but with a five-month gestation from getting a pregnancy started to the point of verifying that you have the product desired in the milk is typically about 18 months.

#### **How much milk is produced per goat per day?**

A goat milks about two liters a day as a typical nominal average figure for a goat. And let me put that in context as terms of what that means.

The precise answer depends on exactly the protein that's there and exactly the productivity of an animal, but just using nominal figures, about a hundred goats can produce about 300 kilograms of pharmaceutical product a year-now 300 kilograms of product that's bulk product. When you put it through purification which typically has about a 50% yield to get to the final injectable form of the molecule, so that would take you to about 150 kilograms of product a year.

There are only about two or three products in the world that are over 150 kilograms from annual production requirement a year. So a hundred goats is what would be necessary to support most products.

#### **How do the levels of protein production compare in goats and traditional bioreactors?**

There are two ways of addressing the question of comparing expression rates in traditional bioreactors or goats. In the straightforward way, the way you asked the question, is that a typical production rate in a bioreactor is 600 milligrams per liter of product. In a goat, a typical range will be on the order of let's say 2 to 8 grams per liter.

But the second point to make here is you're really talking about a completely different apples-and-oranges comparison. That expression rate is extremely critical in a bioreactor because you have a fixed wall and it's a batch process. In

goats it's less critical, to be honest, because regardless of what the expression rate is, you simply breed more animals to get to the number you need to produce the quantity you want, and you're producing it continuously vs. depending upon a batch and the inherent risks of losing that batch.

So there are really two answers here. Number one, transgenics is somewhat better in expression rate typically, and number two, you have the ability to operate it continuously-and if you want to think of it this way-with a flexible walled bioreactor, you can expand it as necessary.

Now having said that, I do believe the bioreactors will get better. They have been getting better. They should get better. People are paying more and more attention because protein-based products are becoming a much more important part of the therapeutic marketplace. They typically go to chronic diseases like arthritis, cancer, AIDS. Many of these diseases are endemic of older populations. Now the young population doesn't typically have a lot of arthritis in it. The industrialized world is aging, and it has the resources to invest to develop products to address these chronic conditions.

Protein-based drugs tend to be able to get there, and since it's a chronic condition you tend to need to have large volumes. You have relatively high doses for the rest of the patient's life, and that leads to very large volumes, and that's the problem the current production system gets into. Even with higher expression rates, you're still talking about very expensive facilities to build, and there isn't a lot of open capacity in existing bioreactors to accommodate the new products.

#### **How is the milk collected?**

As you walked around the farm and you've taken some pictures, it looks very much like a farm here, and in some ways it is. But it's really a biopharmaceutical production facility. What that means is all the way from the ground up, when we first even identified the land, we've developed the sites specifically in mind to getting to pharmaceutical grade products. That means simple things like everything is done by a standard operating procedure, including the milking. That we know everything that comes in contact with the goats: their feed, their water, their bedding, any hay, saltlicks, toys, anything-and there are goat toys by the way-anything that the goat comes in contact with is specified by us and is verified by us to meet those specifications, so we have a high degree of assurance that we have a clean herd and it stays healthy.

Once the animal, or a group of animals, is milking, they're milked in much the same way that a modern dairy farm works. It's a pneumatic milking machine that collects the milk into a central vat, and then the milk is processed. It's also tested and verified.

And one of the first things we do is take all the milk parts out of it. We have a process that we call "tangential flow filtration." It gets to what's known as a "clarified bulk." What I mean by clarified bulk is it's partially purified but not to clinical grade yet, not to the point where it can be introduced to a person.

About half the volume is taken out-all the fats, all the casein, all the whey-and it's no longer white. You end up with an amber, transparent sort of fluid. That then is processed after that point much the way any biologic product is processed today, and that's through chromatography to get to the final medicinal grade product.

Throughout that process just again, as you would do out of any traditional bioreactor product, you have various purification and pathogen removal steps to ensure that if by some odd chance something infected the stream, we know we can take it out and get to a medicinal grade product.

#### **What is chromatography?**

Chromatography is a chemical-based process. It uses what are known as "columns," with media inside of the columns that either repel something or attract something, and you have a series of these columns and they're designed to specifically attract or repel specific items. And you put the fluid over those columns, and by the end of the day you end up with your purified medicinal product.

### **How do the proteins you make in goats differ from human proteins?**

Every biologic product is produced somewhat differently, whether it's produced in a human, a goat, a cow, or a traditional bioreactor. They will be produced slightly differently.

The advantage of mammalian production is that the basic protein structure itself-what I'm talking about are the amino acids, their sequence, and how they're physically structured-is very much the same. The difference is in what's known as the sugars or carbohydrates-what the scientists like to call the "glycosylation pattern." The glycosylation pattern in each biologic system will be different. The issue isn't, "Is it different?" The answer to that is "Yes." The issue is, "Does it mean anything?"

Now to some degree, there are really two issues that you're concerned about with glycosylation. One is when it's given to a person, will the person's immune system react to it and try to kill it, quote unquote, by having a reaction, or will you end up with a material that doesn't have a sufficient half life? It gets cleared out by the liver too fast so that you don't have a practical medicine.

For the most part, we've determined that that's not a problem for transgenic mammalian production just as it's typically not a big problem for bioreactor-based materials that have had to face exactly the same issue.

Now, let's assume for a moment that you do run into a glycosylation pattern that's a challenge. Does that mean you can't do it? Well, typically the answer is no. You still have options. One is if we know that there's a glycosylation pattern early on we have some flexibility in making this DNA construct to try to adjust the glycosylation pattern. That's one way to try and address it.

Another way is, after the protein's been made and the mammary gland comes out, we can do what's known as "post-translational modification." That's typically the use of some sort of an enzyme to either add a sugar or snip a sugar off or perhaps snip the entire sugar off so you just have the base protein. And in that way you can attempt to treat the molecule to get to the kind of profile that you need. But the main basic message is that each biologic system does make protein slightly differently in the glycosylation, and that's usually not a showstopper. You usually have options to try to address that.

### **What other proteins do you produce?**

We actually have a broad range of projects that we're working with. Some of our projects get to very large volumes. What I mean by very large volumes is over 250 kilograms to a metric ton is a pretty large volume product, versus, let's say, nominally a 50-kilogram product is probably a typical sort of a production number for today's protein therapeutics.

Other kinds of products that are difficult to express just aren't made today and the best example of that is actually the plasma proteins, like antithrombin-3. The reason there's a whole plasma marketplace is because these are proteins that have therapeutic value that cannot be made in a recombinant system, so what people have been doing is they've collected human blood, they take that blood and they fractionate it basically to get to the proteins of interest and provide that to doctors for medicinal use.

If you have a recombinant version of the protein, you're basically in a position to offer, number one, a very much more consistent source because the human blood supply doesn't necessarily have the same donors in it at each point in time and, number two, a much more stable source supply, because the human blood supply quite frankly is quite variable. The demand varies and so does the raw supply.

So the advantage of trying to do that in a system that can produce it recombinantly-and transgenic is a system that can do it- is getting to that stable source of supply for a consistent product. And that's what we're able to do in the AT3 program. It's not only the lead program for all transgenic companies, but it's also a very good lead example of getting to these difficult to express proteins.

#### **What are your policies on animal welfare?**

Yes we do use animals here for the purpose of getting two products that help people get to better medical conditions. But concurrent with that objective is our objective to treat these animals in the best way possible, and hopefully as you've seen, as you've toured the facilities, these animals are very well treated. Goats are very social animals. We keep them in social settings. Goats like to nuzzle, push each other. They like to push objects on the ground. They like to climb. We provide those activities for them. We keep them in a very clean environment. We know what other animals may or may not come in contact with them. We monitor their health very well. In fact, we probably have a more detailed health history on each animal here than I have on my own children.

So it's an environment where the animals are very healthy. The kind of life they lead, if you allow me the liberty, is very much a Club Med for goats. If they want to be outside, they can be outside. If they want to play, they can play. If they just want to lie around, they can just lie around, and they can be with the kinds of other goats they want to be with in a social setting.

So it's what we think is a very supportive environment for the animals. We do use them in a milking situation, but the milking situations we put them in are certainly no more threatening than it would be in any agricultural setting and perhaps less. That's up to you to judge.

#### **What are the long-term effects on the goats?**

Our AT3 program has been in goats now for a very long time. It's gone through many generations. I believe we've gone through eight different cycles. The productive life of a goat is typically anywhere between six or seven years. We certainly have not seen any adverse effects among the goats, either within a goat's lifetime or in its succeeding generations.

We've identified that the transgene is stable. What I mean by that is not only from milking cycle to milking cycle in an individual animal, but in, again, the succeeding daughters. You're getting the same gene, the same way, and the same resulting protein in the same way all the time. That's why we have a stable production system, and that's why we believe we have healthy animals here.

There's nothing in the science that tells us there should be a problem. We are inserting a single gene in, such that the desired protein is produced in the animal's milk. It's stable, it stays there but it's still just a goat. It's not a hybrid, it's not a humanoid, it's not a mixed breed. It is still a goat.

#### **What do you see for the future of transgenic technology?**

One of the unique features of the technology is the ability to get to these hard to express proteins. Most investors-and biotech is driven to a large degree by its investor base-are focused on the economics around the technology, and that is a

good story. Getting to these hard to express proteins actually is quite enabling from a drug discovery standpoint.

An example from our own pipeline of products is the malaria vaccine. Now malaria is a devastating disease. It is perhaps the number one disease in the world. It's certainly the number one killer of children. Unfortunately, it's also a disease that happens to be in the third world, where there's not a lot of money to try to address the problem.

There's a malaria vaccine concept the people have had for a long time using a protein called MSP1. It has the potential to be what's known as a therapeutic vaccine and actually treat people who already have the disease with this vaccine. The problem has been that nobody has been able to make the MSP1 protein in significant quantities by which to make an actual product out of it.

We have tested it in monkeys in what's known as the "native form," so the monkey may have already had malaria or may not have. It's been given this form of a vaccine, and it's been successful in staving off what's known as a challenge where we purposely introduced malaria to the monkeys and they were successful in not getting sick.

So this is a very promising concept that can't be done in any other way and because of the power of the economics of the technology has the potential to address it in those areas of the world that can't afford to pay a lot.

#### **Is this research cost effective?**

We're seeking funding for this malaria vaccine from both the Gates Foundation and the National Institutes of Health. We're optimistic that one source or another will fund this. When it's funded, we'll be able to bring it into this facility, which we consider our commercial production facility in goats. And this is an interesting product in that even though it's a very prevailing disease, we probably can meet the world's demand with a handful of goats, probably less than a dozen to meet worldwide demand for our potential malaria vaccine.

#### **Why use goats?**

The reason we would do it in goats is you can't do it in a bioreactor, and goats are a good milking animal. We've done it in transgenic mice, but it's difficult to produce any significant volumes out of a mouse. Mice are a very nice research tool to see whether something is possible, but they're not a good production platform. Goats are a very good production platform.

#### **How many people do you expect this will help?**

I believe the latest figures that I'm aware of malaria affects about 300 million people, and with fewer than a dozen goats we have the potential to being able to treat all of them.

#### **Do you have any final thoughts to share?**

I think the best picture to walk away with here is that this is a technology that is at commercial scale today. It's well demonstrated across a broad range of proteins. We are on the verge of getting regulatory oversight approvals in place, both in Europe and the FDA, and coming on stream as a commercial company. And while there is money involved with commercial-that's sort of the root of the word "commercial"-the exciting thing for many people, including ourselves, is the ability to reach out and make a difference in protein-based therapeutics, especially the hard to express proteins.

#### **Where are you with clinical trials?**

Antithrombin 3 is being studied in the hereditary deficiency condition, those

people that don't make enough of it. It has gone through a pharmacokinetic study. That's just a big word that verifies some of the issues we talked about around glycosylation, that people are not getting any negative response and that the half life is reasonable. It's about to enter the last efficacy stage trial. Efficacy will ensure that the people who need it when they get it don't develop blood clots. That trial should take on the order of a year, roughly, and at that point we'll be ready to file in Europe for approval.

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