Biodistribution of Pfizer Lipid Nano Particles

Dr. Byram Bridle, University of Guelph

Alex talks with Dr. Byram Bridle, an Associate Professor on Viral Immunology at the University of Guelph about a new peer-reviewed study that suggests there may be terrifying reasons side effects such as heart inflammation, VITT, and other serious issues may occur in those who have been vaccinated.

Alex: you've been very, you know, very open on this whole issue, and you're not anti vaccine by any stretch. But what do you think about this? Inflammation in the heart and is it an actual threat?"
Dr Bridle: I'm very much pro vaccine, but always making sure that the science is done properly and that we follow the science carefully before going into public rollout of vaccines. – I'll forewarn you and your listeners that the story I'm about to tell is a bit of a scary one.

The corona virus has a spike protein on its surface. That spike protein allows to infect our bodies. That is why we've been using the spike protein in our vaccines. The vaccines we're using get the cells in our body to manufacture that protein.
If we can mount immune response against that protein, in theory we can prevent this virus from infecting the body. That's the theory behind the vaccine, however, when studying the disease severe COVID-19, everything that you described earlier, heart problems, lots of problems with cardiovascular system, bleeding and clotting is also related with severe COVID-19.

What has been discovered by scientific community is the spike protein on its own is almost entirely responsible for the damage the cardiovascular system.

If you inject the purified spike protein into the blood of research animals, they get all kinds of damage the cardiovascular system, it can cross the blood brain barrier and cause damage to the brain. Now at first glance, that doesn't seem too concerning because we're injecting these vaccines into the shoulder muscle. The assumption until now has been that these vaccines behave like all of our traditional vaccines; that they don't go anywhere, they stay in our shoulder. Some of the protein will go to the local draining lymph node in order to activate the immune system.

However, this is where the cutting edge science has come in, and this is where it gets scary. Through a request for information from the Japanese Regulatory agency, myself and several international collaborators we have been able to get access to what's called the Bio-distribution study. It's the first time ever that scientists have been privy to seeing where these messenger RNA vaccines go after vaccination.
Is it a safe assumption that it stays in the shoulder muscle? The short answer is absolutely not, It's very disconcerting.

The spike protein gets into the blood, circulates through the blood post-vaccination for several days, it accumulates in the blood, and accumulates in a number of tissues, such as the spleen, bone marrow, the liver, the adrenal gland and of particular concern for me is it

accumulates in the ovaries, in quite high concentrations.

Another scientific paper just accepted for publication about 13 young health care workers that received the Moderna vaccine, confirmed that they found the spike protein in circulation in 11 of them.

What this means is: we have known for a long time that the spike protein is a pathogenic protein. It is a toxin that can cause damage in our body if it gets into circulation. Now we have clear cut evidence that when in circulation, the spike protein can do one of two things that can either cause platelets to clump that can lead to clotting. That's exactly why we've been seeing clotting disorders associated with these vaccines. That's why we're seeing heart problems. The protein can also cross the blood brain barrier and cause neurological damage.

The following has not yet been accepted for publication:
They were trying to show that the antibodies from the vaccine get transferred through breast milk and the idea was this may be a good thing, 'cause it would confer some passive protection to babies. However, what they found inadvertently was that the vaccines actually get transferred through the birth breast milk; the vaccine vector itself. Any proteins in the blood will get concentrated in breast milk. Looking into the adverse event database in the United States, we have found evidence of suckling infants experiencing bleeding disorders in the gastrointestinal tract. This has implications for *blood donation* right now. We don't want transfer of these pathogenic spike proteins to fragile patients being transfused with that blood.

We thought the spike protein was a great target antigen, we never knew the spike protein itself was a toxin. By vaccinating people we are inadvertently inoculating them with a toxin.

I have many other legitimate questions about the long term safety.

source: https://omny.fm/shows/on-point-with-alex-pierson/new-peer-reviewed-study-on-covid-19-vaccines-sugge

EMA Report

The following information is from

https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

See p45 Section 2.3.2 Pharmacokinetics

2.3.2. Pharmacokinetics

The applicant has determined the pharmacokinetics of the two novel LNP excipients ALC-0315 (aminolipid) and ALC-0159 (PEG-lipid) in plasma and liver as well as their elimination and metabolism in rats. Furthermore, the Applicant has studied the biodistribution of the two novel lipids (in rats) and the biodistribution of a LNP-formulated surrogate luciferase RNA in mice (IV), as well as the biodistribution of a [3H]-Labelled Lipid Nanoparticle-mRNA Formulation in rats (IM).
No traditional pharmacokinetic or biodistribution studies have been performed with the vaccine candidate BNT162b2. A slower clearance rate was observed after 24 hours with ALC-0315 and ALC-0159 terminal elimination t½ of 139 and 72.7 h, respectively.

In study PF-07302048_06Jul20_072424, the applicant has used a qualified LC-MS/MS method to support quantitation of the two novel LNP excipients. The bioanalysis methods appear to be adequately characterized and validated for use in the GLP studies.
PK studies with the two novel LNP-excipients ALC-0315 and ALC-0159:
Wistar Han rats were IV bolus injected with LNP formulated luciferase-encoding RNA at 1 mg/kg and ALC-0315 and ALC-0159 concentrations at 15,3 mg/kg and 1,96 mg/kg respectively. ALC-0315 and ALC-0159 levels in plasma, liver, urine and faeces were analysed by LC-MS/MS at different time-points up to 2-weeks.

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ALC-0315 and ALC-0159 were rapidly cleared from plasma during the first 24 hours with an initial t½ of 1.62 and 1.72 h, respectively. 24 hours post-dosing, less than 1% of the maximum plasma concentrations remained.
Following plasma clearance, the liver appears to be to major organ to which ALC-0315 and ALC-0159 distribute. The applicant has estimated the percent of dose distributed to the liver to be ~60% for ALC0315 and ~20% for ALC-0159. The observed liver distribution is consistent with the observations from

the biodistribution study and the repeat-dose toxicology, both using IM administration.

For ALC-0315 (aminolipid), the maximum detected concentration in the liver (294 µg/g liver) was reached 3 hours after IV injection. ALC-0315 was eliminated slowly from the liver and after 2-weeks the concentration of ALC-0315 was still ~25% of the maximum concentration indicating that ALC-0315 would be eliminated from rat liver in approximately 6-weeks. For ALC-0159 (PEG-lipid), the maximum detected concentration in the liver (15.2 µg/g liver) was reached 30 minutes following IV injection. ALC-0159, was eliminated from the liver faster than ALC-0315 and after 2-weeks the concentration of ALC-0159 was only ~0,04% of the maximum detected concentration. The applicant was asked to discuss the long half-life of ALC-0315 and its effect, discussion on the comparison with patisiran, as well as the impact on the boosts and post treatment contraception duration. The applicant considered that there were no non-clinical safety issues based on the repeat dose toxicity studies at doses (on a mg/kg basis) much greater than administered to humans; this was acceptable to the CHMP.

Both patisaran lipids showed an essentially similar PK profile in clinic with a strongly biphasic profile and long terminal half-lives. According to the applicant, it is difficult to further contextualize the pharmacokinetic data and therefore to understand the safety of these molecules, beyond consideration of dose. There is a large dose differential between the human BNT162b2 dose and the dose used in the toxicity studies (300-1000x) which provides an acceptable safety margin.

Moreover, according to the Applicant given the large difference in dose between the toxicity studies and the clinically efficacious dose (300-1000x), it is unlikely that the administration of a booster dose will lead to significant accumulation. Finally, the applicant is of the opinion that these results support no requirements for contraception. The CHMP found this position agreeable.

While there was no detectable excretion of either lipid in the urine, the percent of dose excreted unchanged in faeces was ~1% for ALC-0315 and ~50% for ALC-0159.

Biodistribution of a LNP-formulated luciferase surrogate reporter:

To determine the biodistribution of the LNP-formulated modRNA, the applicant did study distribution of the modRNA in two different non-GLP studies, in mice and rats, determined the biodistribution of a surrogate luciferase modRNA formulated with a LNP with identical lipid composition used in BNT162b2 (mouse study) or the biodistribution of a [3H]-Labelled Lipid Nanoparticle-mRNA Formulation (rat study).

The mouse study used three female BALB-c mice per group and luciferase protein expression was determined by in vivo bioluminescence readouts using an In Vivo Imaging System (IVIS) following injection of the luciferase substrate luciferine. The readouts were performed at 6h, 24h, 48h, 72h, 6d and 9d post IM injection (intended clinical route) in the right and left hind leg with each 1 µg (total of

2μg) of LNP-formulated luciferase RNA.

In vivo luciferase expression was detected at different timepoints at the injection sites and in the liver region indicating drainage to the liver. As expected with an mRNA product, the luciferase expression was transient and decreased over time. Luciferase signals at the injection sites, most likely reflecting distribution to the lymph nodes draining the injection sites, peaked 6h post injection with signals of

approximately 10 000 times of buffer control animals. The signal decreased slowly during the first 72 hours and after 6 and 9 days the signals were further weakened to approximately levels of 18 and 7 times the signals obtained from animals injected with buffer control.

The signals from the liver region peaked 6h post injection and decreased to background levels 48h after injection. The liver expression is also supportive of the data from the rat PK study and the findings in the rat repeat-dose toxicological study showing reversible liver vacuolation and increased γGGT levels.

The biodistribution was also studied in rats using radiolabeled LNP and luciferase modRNA (study 185350). The radiolabeling data, measuring distribution to blood, plasma and selected tissues, of IM injection of a single dose of 50 μg mRNA over a 48-hour period is considered more sensitive than the bioluminescence method and indicate a broader biodistribution pattern than was observed with bioluminescence. Over 48 hours, distribution from the injection site to most tissues occurred, with the majority of tissues exhibiting low levels of radioactivity.

Radioactivity was detected in most tissues from the first time point (0.25 h) and results support that injections site and the liver are the major sites of distribution. The greatest mean concentration was found remaining in the injection site at each time point in both sexes. Low levels of radioactivity were detected in most tissues, with the greatest levels in plasma observed 1-4 hours post-dose. Over 48 hours, distribution was mainly observed to liver, adrenal glands, spleen and ovaries, with maximum concentrations observed at 8-48 hours post-dose. Total recovery (% of injected dose) of radiolabeled LNP+modRNA outside the injection site was greatest in the liver (up to 21.5%) and was much less in spleen (≤1.1%), adrenal glands (≤0.1%) and ovaries (≤0.1%). The mean concentrations and tissue distribution pattern were broadly similar between the sexes. No evidence of vaccine-related macroscopic or microscopic findings were found in the ovaries in the repeat-dose toxicity studies (Study 38166 and Study 20GR142) and no effects on fertility were identified in the DART study.

The Japanese Report (referred to by Dr Bryam Bridle)

https://www.pmda.go.jp/drugs/2021/P20210212001/672212000_30300AMX00231_I100_1.pdf

Hard copies of this report have been made and circulated. The tables below are found in the report.

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [^{3}H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159
Report Number: 185350

Sample	Total Lipid concentration (µg lipid equivalent/g [or mL]) (males and females combined)							% of Administered Dose (males and females combined)						
	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h
Lymph node (mandibular)	0.064	0.189	0.290	0.408	0.534	0.554	0.727	--	--	--	--	--	--	--
Lymph node (mesenteric)	0.050	0.146	0.530	0.489	0.689	0.985	1.37	--	--	--	--	--	--	--
Muscle	0.021	0.061	0.084	0.103	0.096	0.095	0.192	--	--	--	--	--	--	--
Ovaries (females)	0.104	1.34	1.64	2.34	3.09	5.24	12.3	0.001	0.009	0.008	0.016	0.025	0.037	0.095
Pancreas	0.081	0.207	0.414	0.380	0.294	0.358	0.599	0.003	0.007	0.014	0.015	0.015	0.011	0.019
Pituitary gland	0.339	0.645	0.868	0.854	0.405	0.478	0.694	0.000	0.001	0.001	0.001	0.000	0.000	0.001
Prostate (males)	0.061	0.091	0.128	0.157	0.150	0.183	0.170	0.001	0.001	0.002	0.003	0.003	0.004	0.003
Salivary glands	0.084	0.193	0.255	0.220	0.135	0.170	0.264	0.003	0.007	0.008	0.008	0.005	0.006	0.009
Skin	0.013	0.208	0.159	0.145	0.119	0.157	0.253	--	--	--	--	--	--	--
Small intestine	0.030	0.221	0.476	0.879	1.28	1.30	1.47	0.024	0.130	0.319	0.543	0.776	0.906	0.835
Spinal cord	0.043	0.097	0.169	0.250	0.106	0.085	0.112	0.001	0.002	0.002	0.003	0.001	0.001	0.001
Spleen	0.334	2.47	7.73	10.3	22.1	20.1	23.4	0.013	0.093	0.325	0.385	0.982	0.821	1.03
Stomach	0.017	0.065	0.115	0.144	0.268	0.152	0.215	0.006	0.019	0.034	0.030	0.040	0.037	0.039
Testes (males)	0.031	0.042	0.079	0.129	0.146	0.304	0.320	0.007	0.010	0.017	0.030	0.034	0.074	0.074
Thymus	0.088	0.243	0.340	0.335	0.196	0.207	0.331	0.004	0.007	0.010	0.012	0.008	0.007	0.008
Thyroid	0.155	0.536	0.842	0.851	0.544	0.578	1.00	0.000	0.001	0.001	0.001	0.001	0.001	0.001
Uterus (females)	0.043	0.203	0.305	0.140	0.287	0.289	0.456	0.002	0.011	0.015	0.008	0.016	0.018	0.022
Whole blood	1.97	4.37	5.40	3.05	1.31	0.909	0.420	--	--	--	--	--	--	--
Plasma	3.97	8.13	8.90	6.50	2.36	1.78	0.805	--	--	--	--	--	--	--
Blood:Plasma ratio[a]	0.815	0.515	0.550	0.510	0.555	0.530	0.540	--	--	--	--	--	--	--

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [^{3}H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159
Report Number: 185350

Species (Strain):	Rat (Wistar Han)
Sex/Number of Animals:	Male and female/3 animals/sex/timepoint (21 animals/sex total for the 50 µg dose)
Feeding Condition:	Fed ad libitum
Method of Administration:	Intramuscular injection
Dose:	50 µg [^{3}H]-08-A01-C0 (lot # NC-0552-1)
Number of Doses:	1
Detection:	Radioactivity quantitation using liquid scintillation counting
Sampling Time (hour):	0.25, 1, 2, 4, 8, 24, and 48 hours post-injection

Sample	Mean total lipid concentration (µg lipid equivalent/g (or mL) (males and females combined)							% of administered dose (males and females combined)						
	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h
Adipose tissue	0.057	0.100	0.126	0.128	0.093	0.084	0.181	--	--	--	--	--	--	--
Adrenal glands	0.271	1.48	2.72	2.89	6.80	13.8	18.2	0.001	0.007	0.010	0.015	0.035	0.066	0.106
Bladder	0.041	0.130	0.146	0.167	0.148	0.247	0.365	0.000	0.001	0.001	0.001	0.001	0.002	0.002
Bone (femur)	0.091	0.195	0.266	0.276	0.340	0.342	0.687	--	--	--	--	--	--	--
Bone marrow (femur)	0.479	0.960	1.24	1.24	1.84	2.49	3.77	--	--	--	--	--	--	--
Brain	0.045	0.100	0.138	0.115	0.073	0.069	0.068	0.007	0.013	0.020	0.016	0.011	0.010	0.009
Eyes	0.010	0.035	0.052	0.067	0.059	0.091	0.112	0.000	0.001	0.001	0.002	0.002	0.002	0.003
Heart	0.282	1.03	1.40	0.987	0.790	0.451	0.546	0.018	0.056	0.084	0.060	0.042	0.027	0.030
Injection site	128	394	311	338	213	195	165	19.9	52.6	31.6	28.4	21.9	29.1	24.6
Kidneys	0.391	1.16	2.05	0.924	0.590	0.426	0.425	0.050	0.124	0.211	0.109	0.075	0.054	0.057
Large intestine	0.013	0.048	0.093	0.287	0.649	1.10	1.34	0.008	0.025	0.065	0.192	0.405	0.692	0.762
Liver	0.737	4.63	11.0	16.5	26.5	19.2	24.3	0.602	2.87	7.33	11.9	18.1	15.4	16.2
Lung	0.492	1.21	1.83	1.50	1.15	1.04	1.09	0.052	0.101	0.178	0.169	0.122	0.101	0.101

The Japanese Findings

After 48 hours, the organs with the highest concentration of the LNPs (**micrograms per ml**) are –

Injection Site	**165**	
Adrenal Glands	**18.2**	**36 x plasma concentration**
Liver	**24.3**	**48 x plasma concentration**
Bone Marrow	**3.77**	**7 x plasma concentration**
Ovaries	**12.3**	**24 x plasma concentration**
Spleen	**23.4**	**46 x plasma concentration**
Large Intestine	1.34	
Small Intestine	1.47	
Lymph Nodes	1.37	

The blood plasma concentration of the LNP after 48 hours is 0.54 micrograms per ml. Therefore the above organs show strong bioaccumulation.

Heart	0.546
Blood	0.54
Brain	0.068

It can be seen that ovaries are exposed to a very high concentration of the lipid nano particles (24 times the concentration found in the blood)

The blood itself contains 0.54 micro grams per ml, so every 2 ml of blood contains 1 microgram of LNP throughout your entire circulatory system.

766.29 g/mol

So 1 ml contains 0.54 x 10^-6/(766.29) x 6.022 x 10^23 = 424 trillion lipid molecules

There are about 5 billion red blood cells in 1ml of blood

So there will be approximately 1000 lipid molecules to every blood cell.

Of course these lipid molecules will be combined together to form Lipid nano particles, but you can see that these might still outnumber the number of red blood cells in your blood.

The Health Worker Report

Dr Bryam Bridle also referred to a report where 11 out of 13 health workers showed significant levels of free-floating spike proteins in their blood for 7 days after vaccination.

This report can be seen here -

https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciab465/6279075?fbclid=IwAR315IPMbov760Vmm7e_qU00op4XaF9TDX1F1IQCbDgysFOc3my23bBKXs8

I have uploaded a hardcopy here –

https://web.facebook.com/groups/352273635911627/permalink/520481879090801

S1 antigen was detected as early as day one post vaccination and peak levels were detected on average five days after the first injection (Figure 1A). The mean S1 peak levels was 68 pg/mL ±21 pg/mL. S1 in all participants declined and became undetectable by day 14.

So the mean spike concentrations in the blood was 68 pg/ml .

This means that each ml of their blood contained 535 million free floating spikes = half a billion spikes

And the total number of spikes within their circulation system was 2.72 trillion. This was the average concentration. There would be locations where the spikes concentrated and bioaccumulated with much greater concentrations..

We know that the mass of the spike protein is 76500 g/mol. So the number of spikes in 68 pg is given by the calculation – 68 x 10^-12/76500 x 6.022 x 10 ^ 23 = 535 million

To put this number into perspective,

- the number of blood cells in 1 ml of blood = 5 billion.
- the number of spikes in 1 ml of the health workers blood was 500 million

And it only takes one of these spikes to trigger clotting.