The Occurrence of Microscopic Vacuoles in Toxicology Studies with Marketed Pegylated Proteins is Associated with High Doses, High Clinical Multiples, and Accumulation

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Introduction and Background

FDA BLA reviews for 9 of 11 pegylated proteins marketed in the US were analyzed to obtain a more complete understanding of microscopic vacuoles in nonclinical toxicology studies.(1) All information was obtained from FDA BLA reviews available on FDA's website and from Package Inserts.

A secondary goal was to structure this analysis to guide development of software tools to analyze large data repositories.

Clinical multiples of PEG doses in repeat-dose toxicology studies were based on cumulative rather than single (nominal) doses because of: 1) the long half-lives of pegylated proteins and drug accumulation in toxicology studies(2); 2) increased dosing frequency in repeat-dose toxicology studies across programs in comparison with recommended clinical dosing schedules; and 3) the relationship across toxicology programs between increased dose and longer study duration with greater anatomic distribution of reported vacuoles, higher vacuole incidence, and less reversibility. Clinical multiples were calculated as cumulative PEG toxicology dose divided by cumulative PEG clinical dose over the same period of time, using clinical doses recommended in Package Inserts. Significant accumulation was not evident at recommended clinical doses.

Plasma or serum levels of drug conjugates across programs were determined with ELISA, activity assays, or hybridization assays, which measured protein levels or activity and generally did not distinguish between pegylated protein fragments or parent conjugate. PEG was not measured.

Vacuoles were reported in nonclinical toxicology studies for Omontys, Cimzia, Macugen, Krystexxa, and Somavert. Anatomic distribution of vacuoles, dose response relationships within these programs and across programs, and clinical multiples were evaluated in this presentation.

Notes:
(1) BLAs for Adagen and Oncaspar were not available.
(2) The exceptions were Pegasys and PegIntron, which were associated with anti-drug antibodies and nonmeasurable drug levels after a few weeks of dosing. Mircera exposure in repeat-dose toxicology studies was consistent with the development of clearance-accelerating anti-drug antibodies. Intravitreal Macugen was associated with low systemic exposure. Vacuoles were not reported in toxicology studies for any of these programs.

MW of PEGs in Pegylated Proteins Marketed in US

Product	Conjugate MW (kD)	PEG MW (kD)	Number of PEG Chains
Cimzia	91	40	1
Krystexxa	540	360	36
Macugen	50	40	2
Somavert	42 – 52	20 – 30	4 – 6
Omontys (1)	45	40	1
Neulasta	39	20	1
Mircera	60	30	1
Pegasys	60	40	2
PegIntron	31	12	1
Oncaspar	483 – 548	345 – 410	69 – 82
Adagen	NA	NA	NA

Mechanistic Grouping of Pegylated Proteins Marketed in US

Mechanism of Action	Product	Protein Portion of Conjugate	Indication	BLA Approval Date
Receptor Agonist or Antagonist	Omontys	ESA	Anemia of CKD	2012
	Mircera	ESA	Anemia of CKD	2007
	Somavert	GHR analog	Acromegaly	2003
	Pegasys	interferon α-2a	HCV	2002
	PegIntron	interferon α-2b	HCV	2001
	Neulasta	G-CSF	CIN	2002
Enzyme	Krystexxa	Uricase	Gout	2010
	Oncaspar	L-asparaginase	ALL	1994
	Adagen	ADA	ADA-SCID	1990
Receptor Ligand	Cimzia	TNFα receptor binding protein	RA, Crohn's	2008
	Macugen	Binds to VEGF	WMD	2004

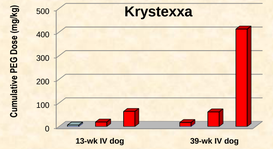
ADA: adenosine deaminase; ALL: acute lymphocytic leukemia; CIN: chemotherapy-induced neutropenia; ESA: erythropoiesis stimulating agent; G-CSF: granulocyte colony stimulating factor; GHR: growth hormone receptor antagonist; HCV: hepatitis c virus; RA: rheumatoid arthritis; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor; WMD: wet macular degeneration.

Anatomic Distribution of Vacuoles at Highest Cumulative PEG Doses and Associated Clinical Multiples

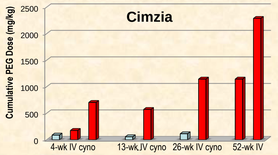
	Omontys	Cimzia	Macugen	Krystexxa	Somavert
Cumulative PEG Dose (mg/kg)	223	2288	728	413	2894
Clinical Multiple (mg/kg)	767	44	6741	54	59
Study Duration (wk)	5	52	13	39	26
Adrenal cortex		✓		✓	✓
Bone marrow			✓		
Choroid plexus	✓	✓			
Heart				✓	
Injection site		✓			✓
Kidney			✓		
Liver (Kupffer cells)			✓	✓	✓
Lymph nodes		✓			✓
Ovary			✓		✓
Pancreas			✓		
Salivary gland			✓		
Small intestine				✓	
Splenic red pulp		✓		✓	✓
Testis			✓		
Uterus		✓			✓

The table above depicts organs in which vacuoles were reported in FDA BLA reviews at the the highest doses in repeat-dose toxicology studies, expressed as cumulative PEG dose (mg/kg). Empty cells indicate that vacuoles were not reported. Pegasys, PegIntron, Mircera, and Neulasta are not included in this table because vacuoles were not reported. Vacuoles were reported to occur in macrophages in the majority of instances. See Introduction and Background for method for calculating clinical multiples and rationale for using cumulative doses.

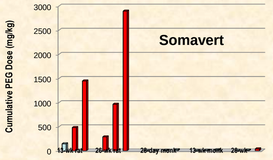
Reported Vacuoles are Dose-related within Studies



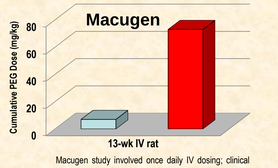
Both studies involved q5d IV dosing. 13-wk study: vacuoles reported in splenic red pulp at cumulative PEG doses ≥19 mg/kg (red) but not 6 mg/kg (blue). 39-week study: dose-related incidence & anatomic distribution of vacuoles reported, all doses, in macrophages in adrenal cortex, liver (Kupffer cells), spleen, small intestine, and/or heart.



All studies involved once-weekly IV dosing. 4-wk study: vacuoles reported in macrophages in spleen, lymph nodes, choroid plexus, ovary, bone marrow, thymus, carotid plexus at ≥176 mg/kg (red). 13-, 26-, & 52-wk studies: vacuoles reported in these tissues plus uterus, injection site at ≥572 mg/kg (red). Vacuole incidences were dose related & partial reversibility was reported (all studies).



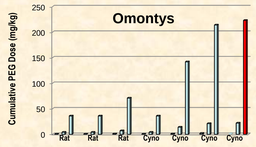
13-wk rat study (daily SC dosing): vacuoles reported in lymph node, injection site at mid dose, these tissues plus liver, adrenal gland at high dose. 26-wk rat study (daily SC dosing): dose-related incidence & distribution of vacuoles in: liver, injection site, lymph nodes, adrenal gland, ovary, spleen, uterus. In monkey studies, vacuoles reported in adrenal cortex at high dose in 26-wk study, once-weekly SC dosing (red).



Macugen study involved once daily IV dosing; clinical route is intravitreal. BLA review states: "phagocytized pegaptinib evidenced by vacuolated macrophages were found in many tissues including bone marrow, kidney, liver, salivary gland, testis, ovary" corresponding to cumulative PEG dose of 73 mg/kg (red).

Vacuoles reported in FDA BLA review

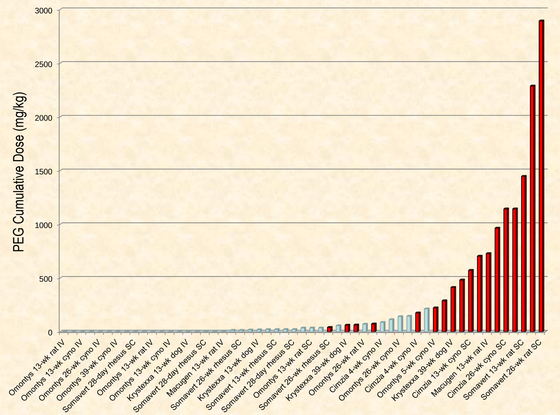
Vacuoles not reported in FDA BLA review



X-axis, from L to R: 13-wk rat IV study, 13-week rat SC study, 26-wk rat IV study, 13-wk cyno IV study, 26-wk cyno IV study, 39-wk cyno IV study, 5-wk cyno SC study. Omontys was given once every 3 weeks in all studies except 5-wk cyno study, which involved 1x/wk dosing. Vacuoles were reported in 5-wk cyno study (high dose).

The graphs above show cumulative PEG doses from all repeat-dose toxicology studies (excluding Macugen, intravitreal route) for Krystexxa, Cimzia, Somavert, Macugen, Omontys. Cumulative PEG doses associated with reported vacuoles are identified by red bars. Cumulative PEG doses for which vacuoles were NOT reported are identified by blue bars. Not included are Pegasys, PegIntron, & Mircera because of decreased exposure/development of anti-drug antibodies & lack of reported vacuoles , and Neulasta, which was associated with low cumulative PEG doses of ≤ 14 mg/kg without reported vacuoles.

Vacuoles were Reported More Frequently with Increasing Cumulative PEG Dose: Analysis Across Studies and Compounds



The graph above is a different way of presenting the same information in the 5 graphs shown in the previous panel to illustrate that reported vacuoles are related to cumulative dose across programs and species.

Vacuoles reported in FDA BLA review

Vacuoles NOT reported in FDA BLA review

Conclusions

1. Microscopic vacuoles have been reported in FDA BLA reviews to occur in repeat-dose toxicology studies in multiple species and with multiple PEGylated compounds.
2. Their reported occurrence appears generally dose related within studies and across programs and unrelated to PEG MW or pharmacologic activity of protein.
3. In some cases, vacuoles were associated with very large multiples of cumulative clinical PEG (and conjugate) dose.
4. In nearly all cases, vacuoles were found in macrophages, often in organs/tissues associated with the macrophage/phagocyte system. Associated functional changes were not reported.
5. Dose selection as outlined in ICH S6 (Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals) should be followed to avoid use of excessively high doses of PEGylated proteins and accumulation.