Toxicogenomic Investigation of Compound-induced GI Toxicity

Proposed Data Submission Content and Format

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Acknowledgments

- Peter Lord and HL7/CDISC/I3C Track 1 Data Standards group
- John Leighton for help in finalizing draft case study

- Purpose: Provide real-world case study as basis for discussion of submission format; sponsor *wants* to submit data from GLP work to support regulatory package
- Example consists of real data* interspersed with contrived data to fill critical gaps
- Intent is to highlight submission issues rather than discuss study design, data analysis, etc.

*Milano et al (2004) Tox Sci, 82, 341-358.
Searfoss et al (2003) J. Biol. Chem., 278, 46107-46116.

Outline of Submission

- Title
- Introduction
- Methods
  - Study design
  - Details of genomics, biochemical work
- Results (Data)
  - Tables, Figures
- Discussion
- References (literature, databases, websites)

Introduction

- γ secretase inhibitors X & Y caused GI lesion in animals w/o margin, halting development; Z did not show lesion @ > efficacious doses, up to MTD; standard safety package supports taking Z into clinic
- Investigations to be reported, of which toxicogenomics is one part, conducted to:
  - differentiate compound Z from X & Y
  - understand potential mechanism of lesion
  - develop biomarker, that is demonstrably mechanism-based, to monitor in clinic

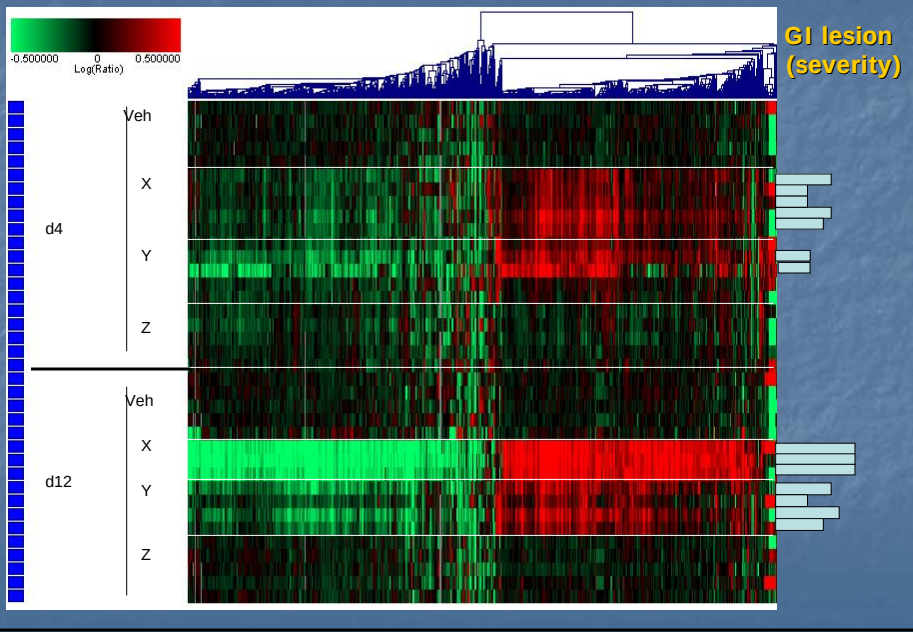
Methods

- Study Design
- Details of expression profiling
  - RNA prep, hybridization
  - Array characteristics
  - Data capture, processing, normalization
  - Analysis package, criteria for filtering
  - Visualization methods
- Details of follow-up work (PCR, biochemistry, etc.)

Data

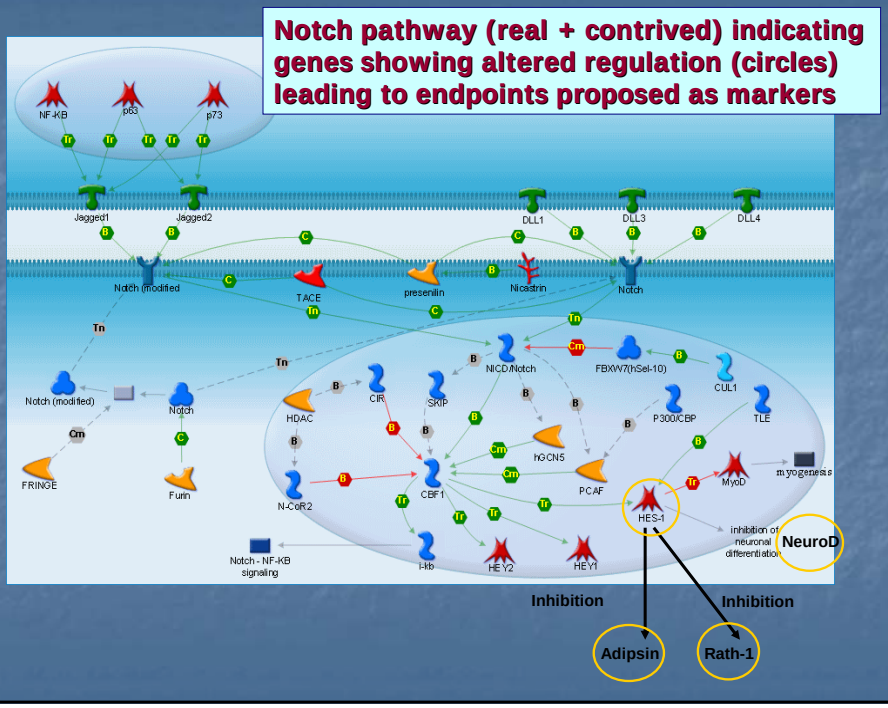
- GI lesion (animal studies) – goblet cell metaplasia, duodenum, assoc w/ diarrhea
  - seen with X, Y @ 100 mpk, as early as d 4; not with Z @ 150 mpk (MTD), day 12
  - Z more efficacious @ 100 than X, Y
- Genomics supports differential response with X, Y compared to Z
- Analysis of genes suggests notch-based mechanism; adipsin, Rath-1 identified as downstream markers of perturbation of notch pathway

Agglomerative cluster; p<0.001, 2-fold change



Data Reporting

"One way ANOVA analysis (Resolver V5.0.0.1.32) was carried out to identify the gene expression changes that differentiate the toxic compounds X and Y from the non toxic compound Z. Two ANOVA sets of genes were generated using 2-fold change, P<=0.001 as filters (see Methods for further details). The files containing these results are Gene List-1 X v Y.xls and Gene List-1 XY v Z.xls, stored in the file repository."



Data (not shown)

- PCR of 4 target genes, immunohistochem, biochemistry support notch pathway involvement (multiple lines of support for hypothesis)
- Adipsin, Rath-1 proteins found in feces only when metaplasia also seen; not found when diarrhea induced non-specifically in non-human primates (specificity) by other agents

Table N*: 81 annotated genes among the 219 genes that differentiate compound X and Y from Z across day 4 and 12

Sequence or Gene		Day 4			Day 12		
Name	Code	Xd4 AFC	Yd4 AFC	Zd4 AFC	Xd12 AFC	Yd12 AFC	Zd12 AFC
Rath-1	NM_007500	3.4801	2.124561	0.1800112	3.00011	3.35001	0.18432
Adipsin	M92059	3.1123465	2.7024351	0.0611205	3.6657631	3.2511	0.1612
Hes1	NM_024360	-1.5835624	-2.2116768	0.2477168	-1.455613667	-2.11506675	0.1819202
Neurod1	NM_019218	5.0764574	3.5569432	1.2268218	3.602745333	3.6453945	1.191378
Pam	600510684R1	0.8717196	0.9340476	-1.1572642	2.949614	1.8043085	-0.2837008
Slc13a2	AB001321	-1.4684788	-1.5770714	-0.686734	-9.262257667	-2.28789875	-1.1801484
Pib5pa	AB032551	1.4171508	1.9772702	-1.120923	1.837448333	2.00085625	-0.2474462
Jdp1	AB062135	1.273224	0.7473766	-1.1278078	2.233502667	1.42685625	-0.5992188
Nupr1	AF014503	1.919542	1.367877	-0.9570788	6.612407333	3.171801	-0.2437412
Zdhhc2	AF228917	1.9409794	1.5964512	-0.6490912	3.666386	1.87568525	0.673965
Xpnpep2	AF359355	-1.2942324	-1.4890574	-0.2304996	-7.647647	-2.29679775	-0.2676778
Gstm2	AI502080	-1.4218198	-1.433535	0.653768	-2.658683333	-0.70656	1.1475466
Abcc4	AW141985	1.3258792	1.2899682	0.2116792	2.211431	1.37927325	0.6642708
Acsl6	D10041	3.5884042	4.0979948	0.695588	4.260271	2.697035	0.745463

***Excel table: Submit to NDA; no known or probable valid biomarkers except first 4, which were experimentally confirmed**

Table N SEND (v2.1 on CDISC) Vocabulary for Toxicogenomics Data Submission (modeled after clinical pathology SEND format)

Toxicogenomics - TG		
Findings - One record per gene per animal (sample)		

Variable Name	Variable Label	Type	Controlled Terms or Format	Origin	Role	Usage Notes
STUDYID	Study Identifier	Char			Identifier	Unique identifier for a study within the submission
USUBJID	Animal Identifier	Char			Identifier	Animal identifier
DOMAIN	Domain	Char	TG		Identifier	Two-character code for the domain of the study, TG - Toxocogenomics
TGREFID	Pool Identifier	Char	CP		Identifier	Unique identifier for a pool of samples (unique if combined with TGDTT). Not used if there is no poolign of samples
TGCPT	Compound Identifier	Char			Identifier	Compound number or identifier
TGSEQ	Sequence Number	Num			Identifier	Sequence number given to ensure uniqueness within a dataset for an animal.
TGOTND	Organ, Tissue Name	Char	OT		Identifier	Unique name for organ or tissue
TGTESTCD	Gene Name	Char			Identifier	Gene name
TGTESTID	Accession Number	Char			Idnetifier	GenBank gene accession number

Table N** SEND (v2.1 on CDISC) format of Toxicogenomics data for Adipsin, Rath-1, Hes-1 and NeuroD

The dysregulation of Adipsin, Rath-1, Hes-1 and Neuro D were listed per animal per compound (X, Y and Z), per time point (day 4 and 12) as Log(Ratio).

Peudo SEND Format

STUDY ID	USUBJID	DOMAIN	TGREFID	TGCPT	TGSEQ	TGNTOD	TGTESTCD	TGTESTID	TGORRES	TGORRESU	TGDTT
100001	234500	TG	CP		1	Duodenum					4
100001	234501	TG	CP		2	Duodenum					4
100001	234502	TG	CP		3	Duodenum					4
100001	234503	TG	CP		4	Duodenum					4
100001	234504	TG	CP		5	Duodenum					4
100001	234505	TG		X	6	Duodenum	Adipsin	M92059	3.3	Log(Ratio)	4
100001	234506	TG		X	7	Duodenum	Adipsin	M92059	3.2	Log(Ratio)	4
100001	234507	TG		X	8	Duodenum	Adipsin	M92059	2.9	Log(Ratio)	4
100001	234508	TG		X	9	Duodenum	Adipsin	M92059	2.8	Log(Ratio)	4
100001	234509	TG		X	10	Duodenum	Adipsin	M92059	3.1	Log(Ratio)	4
100001	234510	TG		Y	11	Duodenum	Adipsin	M92059	2.7	Log(Ratio)	4
100001	234511	TG		Y	12	Duodenum	Adipsin	M92059	2.9	Log(Ratio)	4
100001	234512	TG		Y	13	Duodenum	Adipsin	M92059	2.4	Log(Ratio)	4
100001	234515	TG		Z	16	Duodenum	Adipsin	M92059	0.1	Log(Ratio)	4
100001	234516	TG		Z	17	Duodenum	Adipsin	M92059	0.2	Log(Ratio)	4
100001	234517	TG		Z	18	Duodenum	Adipsin	M92059	-0.1	Log(Ratio)	4
100001	234518	TG		Z	19	Duodenum	Adipsin	M92059	0.1	Log(Ratio)	4
100001	234519	TG		Z	20	Duodenum	Adipsin	M92059	0.1	Log(Ratio)	4
100001	234505	TG		X	6	Duodenum	Hes-1	NM024360	-2.4	Log(Ratio)	4

****SEND table: Submit to IND; only probable valid (experimentally confirmed) on individual animal basis**

Conclusions

- Genomics data support notch-associated mechanism of GI toxicity with X & Y
- Compound Z shows no evidence of lesion at MTD (animal studies); chosen as development candidate; standard IND safety package generated
- Evidence from a battery of studies (not limited to toxicogenomics) suggests adipsin, ath-1 as mechanism-based markers of lesion across species
- These probable valid biomarkers support differentiation of X, Y from Z
- Proposal: monitor adipsin, ath-1 in clinic as qualified leading biomarkers of unexpected GI toxicity w/ Z

Discussion Points

- **Point: If submission provides rationale for clinical biomarker, does it differ from one supporting risk assessment claim?**
  - Standard preclinical package sufficient & pivotal to support safety of compound Z; no intent to address risk with these data, but data can add to weight of evidence
  - Limited purpose for submission, support for proposed clinical markers allowing compound Z to proceed safely into humans

Discussion Points

- **Point:** Toxicogenomics data is only one piece of evidence supporting hypothesis
  - What is expectation of extent of genomics submission ? (all gene sequences, relevant ones, experimentally confirmed genes?)
  - Guidance indicates that all gene changes are not required for IND submission
  - Only "known and probable valid" gene sequences submitted to IND – those interpreted to have biological meaning and whose context was confirmed by follow-up experiments

Backup Slides

Factors Driving IND Data Submission

Study Purpose	Level of Qualification: Low (Validity not established)	Med. (Probable valid)	High (Known valid)
GLP Study w Potential for Data w Regulatory Impact			
Used by sponsor in decision to support the safety of a clinical trial	Vol*	*FuR*	*FuR*
Not used to assess prognosis of animal findings (eg, explore mech.)	Vol*	Vol*	*AbR*
Non-GLP Exploratory Study for Internal Decisions	Vol	Vol	Vol

*If additional information becomes available, sponsor must submit

NDA Data Submission

Study Purpose	Level of Qualification: Low (Validity not established)	Med. (Probable valid)	High (Known valid)
GLP Study w Potential for Data w Regulatory Impact			
Used by sponsor to support a safety claim	*Synop*	*AbR*	*FuR*
Not used to assess prognosis of animal findings	*Synop*	*AbR*	*AbR*
Non-GLP Exploratory Study for Internal Decisions	*Synop*	*AbR*	*AbR*

*314.50 CFR: ..the [NDA] application is required to contain reports of all investigations of the drug product sponsored by the applicant."