Draft NTP Technical Reports on 3'-Azido-3'-Deoxythymidine (GMM-14) and in Combination with Lamivudine and Nevirapine (GMM-16)

Julian Leakey, PhD, DABT

National Center for Toxicological Research

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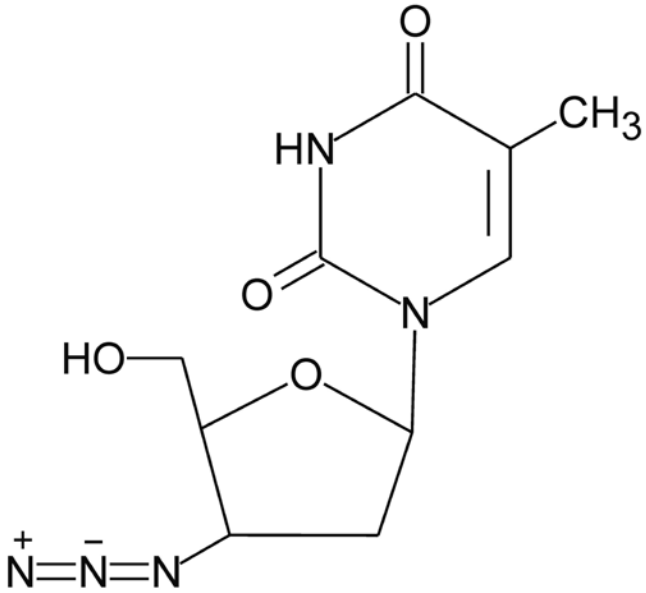
AZT



3’-Azido-3’-deoxythymine,
AZT, Zidovudine

AZT is an analogue of thymidine and was the first NRTI to be used successfully to treat AIDS.

Adult oral doses of 300 mg b.i.d. are required to maintain therapeutic blood levels of 2 – 4 μM.

It is phosphorylated *in vivo* to AZT-TP, which inhibits retroviral transcription.

AZT is still widely used worldwide as part of combination drug therapy required to stop mother-to-child transmission of HIV.

AZT ADME Issues

- Only a small fraction of the dose is phosphorylated to pharmacologically active AZT-TP.
- In humans, AZT is actively glucuronidated and eliminated in the urine predominantly as a glucuronide conjugate.
- Rodents do not actively glucuronidate AZT and eliminate it in urine predominantly as free AZT.
- The elimination rates in mice and humans are similar.

Mechanisms of AZT-Induced Toxicity in Eukaryotic Cells

- AZT-TP is a weak inhibitor of nuclear and mitochondrial DNA polymerases
- AZT-MP accumulates in mitochondria disrupting metabolism and inhibiting mitochondrial production of TTP
- In cells that are not active in DNA replication, mitochondrial TTP is required for nuclear DNA repair

Human Toxicity

- Common side effects of AZT therapy include severe lactic acidosis, macrocytic anemia and muscle wasting.
- These conditions are reversible.
- DNA adduct incorporation and mutations detected in cord blood cells from infants that were transplacentally exposed to AZT.
- The long-term (lifetime) consequences of perinatal exposure to AZT are unknown.

Rodent Toxicity and Carcinogenesis Studies of AZT

- Cardiotoxicity and mitochondrial damage observed in CD-1 mice dosed b.i.d. from gestation until PND 28.
- Macrocytic anemia noted in Industry studies.
- Cancer studies (outlined previously) demonstrated some carcinogenic activity of AZT – particularly at high doses.
- Different tissues affected depending on the rodent strain and conditions of exposure.

Developing the C3B6.129F1*Trp53*^{tm1Brd} *p53* haploinsufficient (+/-) mouse model

Produced by: Mating C57BL6.129[N12]*trp53*$^{(-/-)}$ male mice with Taconic C3H females.

Significance: Haploinsufficient mouse will develop tumors at an increased rate, shortening duration of carcinogenicity studies.

Abbreviated: Heterozygous F1 *p53*$^{+/-}$ Mouse

Comparable to: NTP B6C3F1 except the strains of the male and female parents are reversed. Hence C3B6F1.

Advantages: C3H Dams are tolerant of pup dosing. Similar background tumor incidence to NTP B6C3F1.

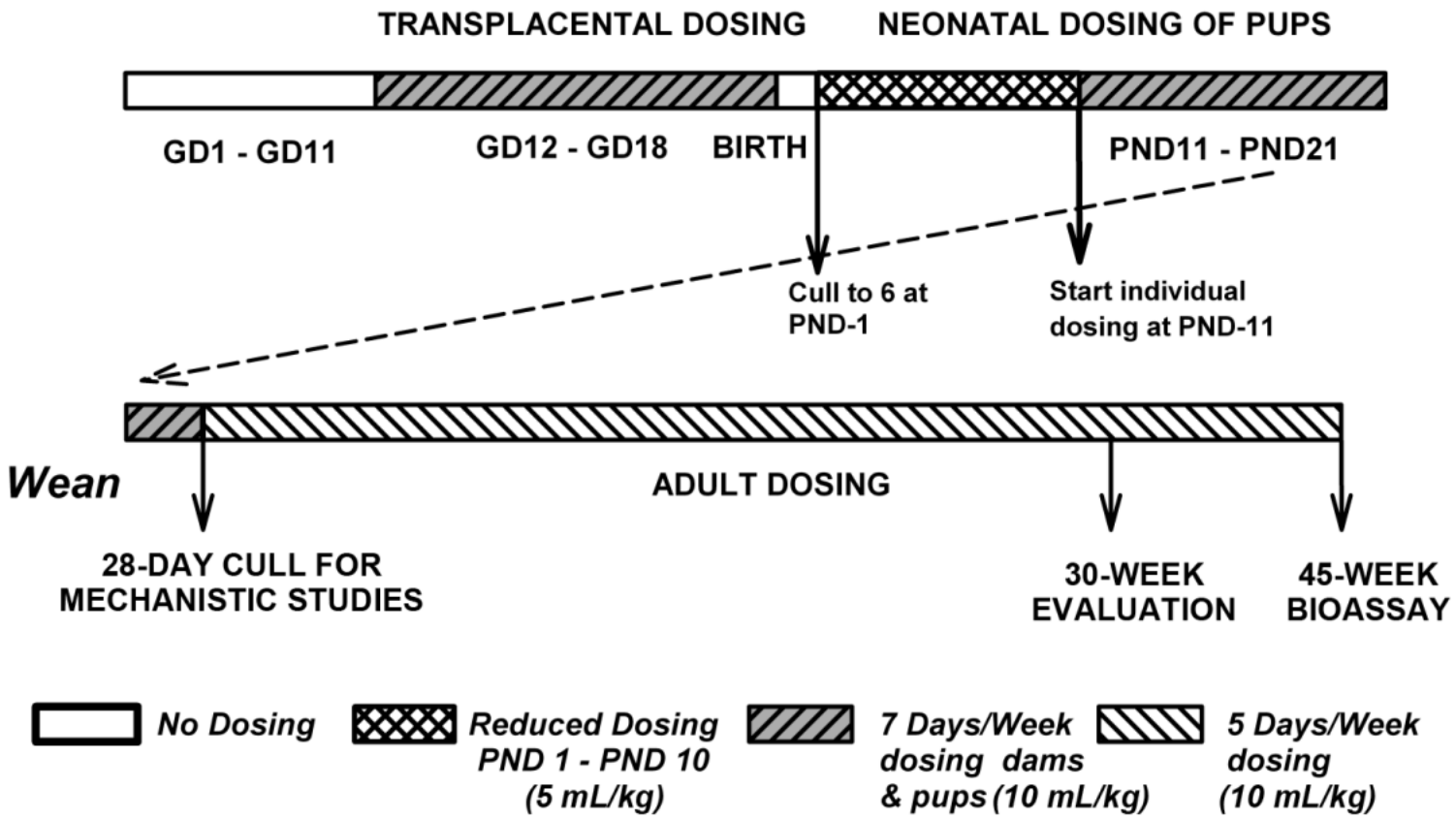
GMM 14 – designed to test whether the model would detect carcinogenicity using AZT alone

- In this study mice were exposed to AZT alone from GD12 until 9 months of age using once per day dosing.
- Transplacental exposure to AZT had resulted in increased incidence of lung tumors in Swiss mice. Tumors exhibited p53 mutations (NTP TR 522).
- AZT exposure increased the incidence of micronucleated reticulocytes in B6C3F1 mice.

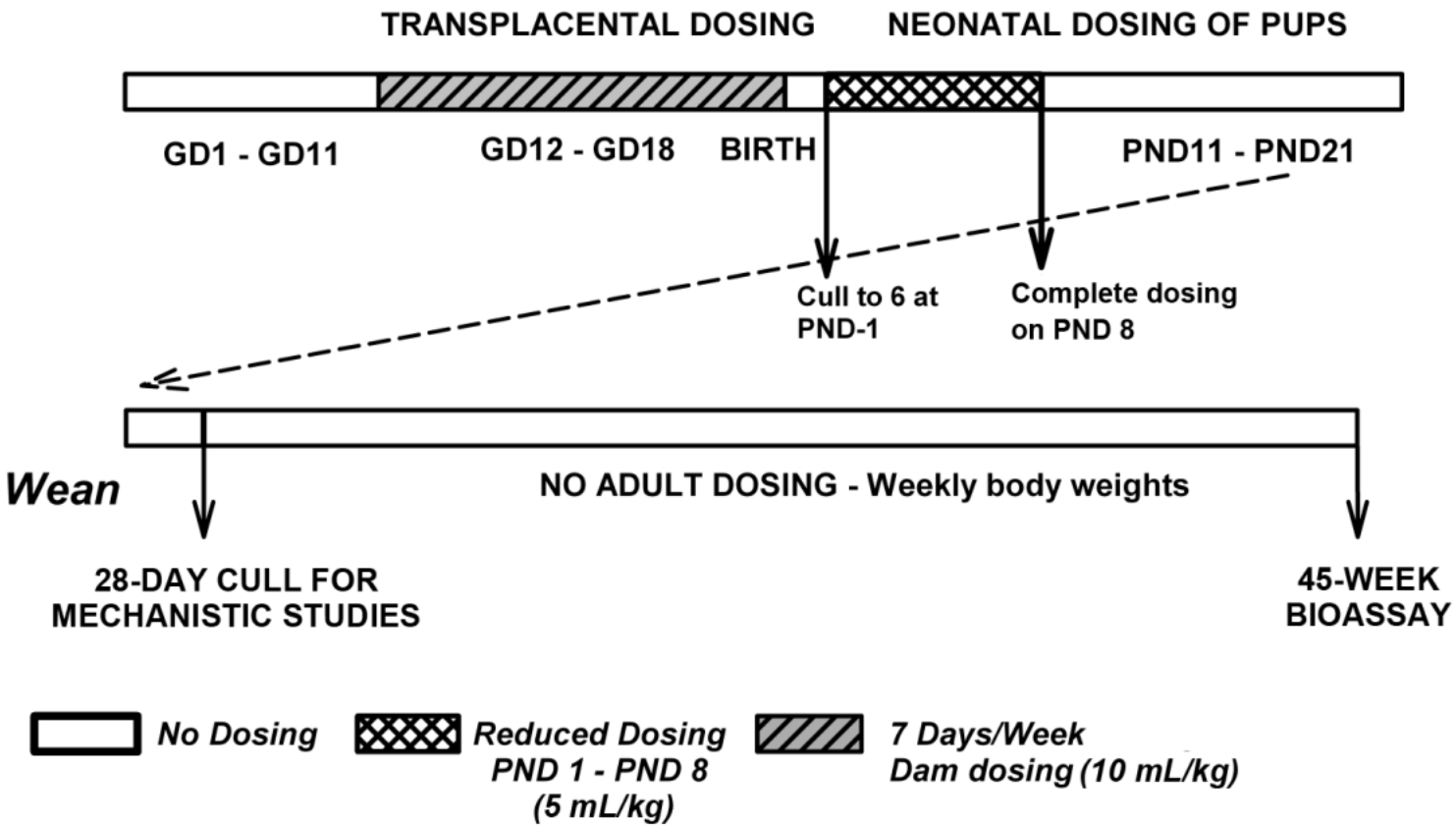
GMM 14 Experimental Design

- 25 – 27 mice per sex in each F1 dose group.
- Dosed via oral gavage, dosing vehicle was 0.1% Tween® 80 and 0.2% methylcellulose in water.
- Main study dosed F1 pups until 45 weeks of age, but included a 30 week interim evaluation.
- Stop study used same doses as ongoing 2-year study with B6C3F1 mice.
- Statistical analysis evaluated potential for lesion clustering within littermates.

GMM 14 Main Study Design



GMM 14 Main Stop-Study Design



GMM 14: Litter parameters and survival to PND 28 for Heterozygous F1 p53$^{(+/-)}$ mice

AZT Dose Groups	*Continuous Dosing*				*Stop Study*	
[mg/kg/day]	0/0/0	80/40/80	160/80/160	240/120/240	0/0	240/40
Total litters	46	18	16	50	-*	-*
Total pups born	314	141	105	351	-*	-*
Average per litter	6.8	7.8	6.8	7.0	-*	-*
Survival PND 1 -10	92.8%	98.1%	97.8%	95.3%	98.6%	96.5%
Survival PND 11 - 28	96.7%	91.1%	98.9%	93.8%	98.6%	100%
# assigned to study	107	54	54	106	50	51
Litters used	34	18	16	36	12	14

Table 3 from GMM 14. * Included with the continuous dosing mice

GMM-14: Effects on body weight and survival

- **Small (<10%), dose-related decreases in body weight in both male and female F1 dose groups [Figs. 4, 6 & Appendix D].**
- **Body weight decreases also observed in the stop-study [Fig. 8, & Appendix D].**
- **No significant dose-effect on survival except for male heterozygous F1 *p53*$^{+/-}$ mice dosed with 240/120/240 AZT and evaluated at 30 weeks, where overall survival was significantly reduced from 100% to 80.8% [Tables 4, 6 & 9, & Figs. 3, 5, 7]**

Malignant neoplasms in male Heterozygous F1 p53$^{(+/-)}$ mice after 30 weeks exposure

Lesion	*0/0/0 mg/kg*	*240/120/240 mg/kg*
Malignant lymphoma: Overall rate	0/27	3/26 (11.5%)
Poly-3 and litter adjusted rate	0.0%	14.4%
Litter adjusted Poly-3 significance	-	P = 0.005

From Table 5 and Appendix G of GMM 14

Malignant lymphoma in female Heterozygous F1 p53$^{(+/-)}$ mice after 45 weeks exposure

Lesion	*AZT Dose Groups (mg/kg)*			
	0/0/0	*80/40/80*	*160/80/160*	*240/120/240*
Malignant lymphoma				
Overall Rate	0/26 (0.0%)	0/27 (0.0%)	1/27 (3.7%)	3/27 (11.1%)
Poly-3 Adjusted Rate	0.0%	0.0%	4.0%	12.2%
First Incidence (days)	-	-	322T	241
Poly-3 Test	**0.020***	-	0.505	0.115
Adjusted Poly-3 Test[a]	**0.023***	-	0.148	**0.033***

Table 7 from GMM 14. Historical Incidence = 3/102. *Significant (P< 0.05)

[a]Adjusted for litter cluster correlation (Appendix G)

Hepatocellular neoplasms in male Heterozygous F1 p53(+/-) mice after 45 weeks exposure

Lesion	*AZT Dose Groups (mg/kg)*			
	0/0/0	*80/40/80*	*160/80/160*	*240/120/240*
Liver Hepatocellular Adenoma				
Overall Rate	3/26 (11.5%)	2/27 (7.4%)	6/27 (22.2%)	9/27 (33.3%)
Poly-3 Adjusted Rate	12.3%	8.8%	22.2%	36.5%
Terminal Rate	3/24 (12.5%)	2/21 (9.5%)	6/27 (22.2%)	7/22 (31.8%)
First Incidence (days)	321T	319T	320T	228
Poly-3 Test	**0.013***	0.531N	0.288	**0.048***
Adjusted Poly-3 Test[a]	**0.010***	0.214N	0.168	**0.027***
Liver Hepatocellular Adenoma or Carcinoma				
Overall Rate	3/26 (11.5%)	3/27 (11.1%)	6/27 (22.2%)	9/27 (33.3%)
Poly-3 Adjusted Rate	12.3%	13.2%	22.2%	36.5%
Terminal Rate	3/24 (12.5%)	3/21 (14.3%)	6/27 (22.2%)	7/22 (31.8%)
First Incidence (days)	321T	319T	320T	228
Poly-3 Test	**0.019***	0.634	0.288	**0.048***
Adjusted Poly-3 Test[a]	**0.016***	0.417N	0.174	**0.029***

Table 7 from GMM 14. Historical Incidence 8/100 *Significant (P< 0.05)

[a]Adjusted for litter cluster correlation (Appendix G)

Hepatocellular neoplasms in male Heterozygous F1 p53$^{(+/-)}$ mice: Stop-study

Lesion	0/0/0 mg/kg	240/40 mg/kg
Hepatocellular adenoma		
Overall Rate	3/24 (12.5%)	5/24 (20.0%)
Poly-3 Adjusted rate	12.8%	21.3%
First incidence (Days)	317T	317T
Poly-3 Test	0.352	-
Litter-Adjusted Poly-3 Test	0.212	-
Hepatocellular Adenoma or carcinoma		
Overall rate	3/24 (12.5)	7/25 (28%)
Poly-3 Adjusted Rate	**12.8%**	**29.8%**
Poly-3 Test	0.143	-
Litter-Adjusted Poly-3 Test	0.076	-

From Appendix G GMM 14. Historical Incidence 8/100.

GMM 14: Significant Observations

- **The heterozygous F1 *p53*$^{+/-}$ mouse model detected treatment-related carcinogenesis within 12 months of initiation of exposure.**
- **Treatment-related tumor profile similar to B6C3F1 mouse.**
- **Under the conditions of the study transplacental and neonatal dosing was well tolerated.**
- **No evidence of lesion-clustering within littermates (Appendix G).**

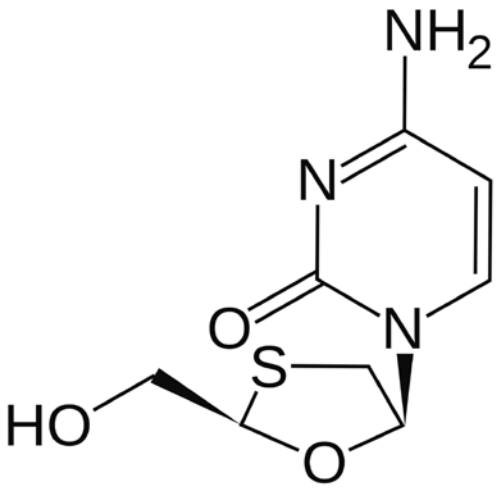
Conclusions – GMM 14

- **Under the conditions of these gavage studies, there was *clear evidence* *of carcinogenic activity* of AZT in male heterozygous F1 p53$^{+/-}$ mice based on the occurrence of hepatocellular neoplasms (predominantly adenomas) after 45 weeks of administration. The occurrence of malignant lymphoma may have been related to AZT administration for 30 weeks.**
- **There was *equivocal evidence* *of carcinogenic activity* of AZT in female heterozygous F1 p53$^{+/-}$ mice based on the occurrence of malignant lymphoma after 45 weeks of administration.**

GMM 16 – designed to test AZT in combination with 3TC and NVP

- **Follow-up study dosed mice with AZT, 3TC and NVP alone or in combination from GD12 until PND 28, using twice per day dosing.**
- **Designed to more closely mimic the clinical situation where infants are dosed with drug combinations (b.i.d.), but only prenatally and in infancy.**

Lamivudine (3TC)



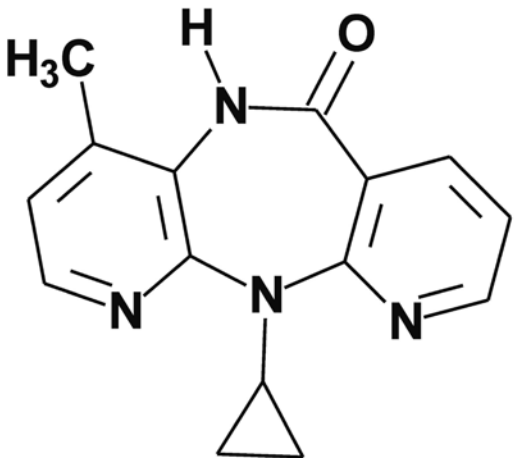
2',3'-Dideoxy-3'-thiacytidine, 3TC, lamivudine, *Epivir.*

3TC is a NRTI used to treat AIDS, which is marketed in combination with AZT as *Combivir.*

It is prescribed with AZT to inhibit mother-to-child transmission of HIV.

Although it is relatively non-toxic, it has been shown to increase the frequency of micronuclei in reticulocytes of mice exposed to 3TC together with AZT.

Nevirapine (NVP)



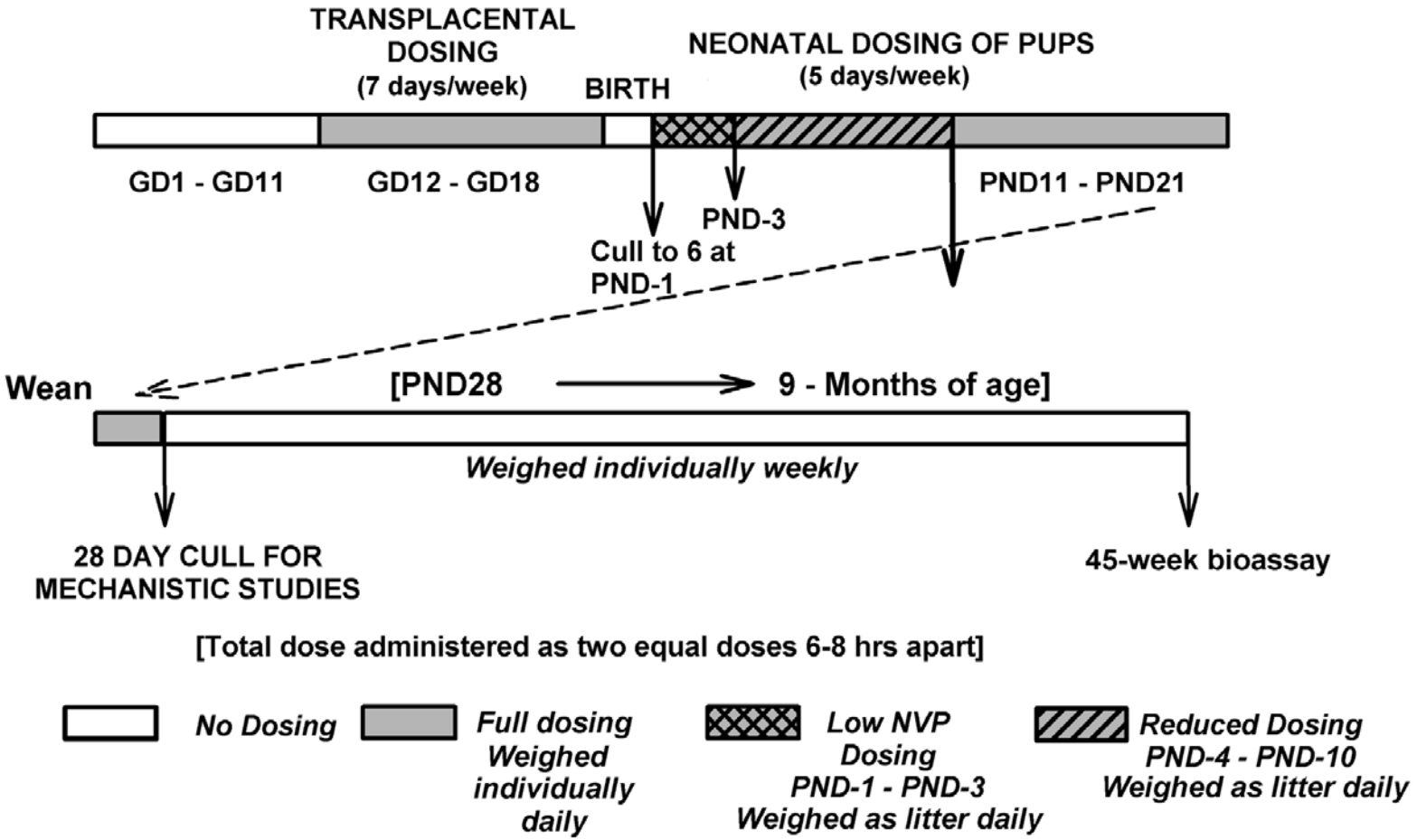
Nevirapine, *Viramune*

NVP is a NNRTI that is given in conjunction with AZT and other drugs to treat AIDS. It is generally given as a short term treatment, because its metabolites form protein adducts that can cause hypersensitivity reactions.

NVP is a CAR agonist – CYP2B inducer and a rodent hepatocarcino-gen when administered chronically.

NVP did not increase micronucleus frequency in mouse reticulocytes, but is reported to form adducts with DNA.

GMM 16 Study Design



GMM 16 Dose Groups

Dose Group Name	Fo GD 12 - GD 18	F_1 PND 1 - PND 10	F_1 PND 11 - PND 28
Vehicle Control	Aqueous methyl-cellulose/ Tween 80 **10 ml/kg 2x per Day**	Aqueous methyl-cellulose/ Tween 80 **3 ml/kg 2x per Day**	Aqueous methyl-cellulose/ Tween 80 **10 ml/kg 2x per Day**
AZT-H	240 mg AZT per kg/day	120 mg AZT per kg/day	240 mg AZT per kg/day
3TC-H	150 mg 3TC per kg/day	75 mg 3TC per kg/day	150 mg 3TC per kg/day
NVP-H[a]	168 mg NVP per kg/day	**84** mg NVP per kg/day	168 mg NVP per kg/day
AZT/3TC-H	240/150 mg AZT/3TC per kg/day	120/75 mg AZT/3TC per kg/day	240/150 mg AZT/3TC per kg/day
AZT/3TC/NVP-L[a]	80/50/56 mg AZT/3TC/NVP per kg/day	40/25/**28** mg AZT/3TC/NVP per kg/day	80/50/56 mg AZT/3TC/NVP per kg/day
AZT/3TC/NVP-M[a]	160/100/112 mg AZT/3TC/NVP per kg/day	80/50/**56** mg AZT/3TC/NVP per kg/day	160/100/112 mg AZT/3TC/NVP per kg/day
AZT/3TC/NVP-H[a]	240/150/168 mg AZT/3TC/NVP per kg/day	120/75/**84** mg AZT/3TC/NVP per kg/day	240/150/168 mg AZT/3TC/NVP per kg/day

[a] **NVP component of the dose reduced 4x from PND 1 - PND 3**

Combination dose comparison =
Vehicle Control: AZT/3TC/NVP-L: AZT/3TC/NVP-M: AZT/3TC/NVP-H
High dose comparison =
Vehicle Control: AZT-H: 3TC-H: NVP-H: AZT/3TC-H: AZT/3TC/NVP-H

Litter parameters and survival to PND 28 for Heterozygous F1 p53$^{(+/-)}$ mice: Combination dose comparison

	AZT Dose Groups (mg/kg/day)			
	Vehicle Control	**AZT/3TC/ NVP-L**	**AZT/3TC/ NVP-M**	**AZT/3TC/ NVP-H**
Total Litters	28	24	23	35
Total Pups born	204	180	156	208
Average per litter	7.29	7.5	6.78	**5.94**
Survival PND 1 - 10	82.74%	78.70%	90.58%	81.03%
Survival PND 11 - 28	96.54%	98.02%	81.52%	**48.82%**
Survival analysis*	**<0.001**	-	**0.006**	**<0.001**
# assigned to study	50	50	50	50
Litters used	24	16	16	24

Table 3 from GMM 16. *p values for PND11 - 28.

Litter parameters and survival to PND 28 for Heterozygous F1 p53$^{(+/-)}$ mice: High dose comparison

Lesion	*AZT Dose Groups (mg/kg/day)*					
	Vehicle Control	AZT-H	3TC-H	NVP-H	AZT/3TC-H	AZT/3TC/ NVP-H
Total litters	28	29	25	23	27	35
Total pups born	204	206	147	155	184	208
Average per litter	7.29	7.10	**5.88**	6.74	6.81	**5.94**
Survival PND 1 -10	82.74%	83.10%	86.11%	86.23%	94.00%	81.03%
Survival PND 11 - 28	96.54%	**80.60%**	99.09%	99.24%	**71.47%**	**48.82%**
Survival analysis*	-	**0.037***	-	-	**<0.001**	**<0.001**
# assigned to study	50	50	50	51	50	50
Litters used	24	22	17	19	20	24

Table 4 from GMM 16. *p values for PND11 - 28.

GMM 16 survival and body weight effects

- Survival was 75% or greater across all dose groups for the pups assigned to the study on PND28. There were no significant dose-dependent decreases in survival. In most cases, early deaths were associated with malignant neoplasms.
- There was a dose-dependent decrease in body weight gain in the male mice treated with AZT/3TC/NVP combinations.
- The high dose comparison suggested that all three component drugs contributed to the body weight decrease.

Malignant lymphoma in female Heterozygous F1 p53$^{(+/-)}$ mice: Combination dose comparison

Lesion	*AZT Dose Groups (mg/kg/day)*			
	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Malignant lymphoma (all organs)				
Overall Rate	2/25 (8.0%)	2/25 (8.0%)	4/22 (**18.2**%)	4/25 (**16.0**%)
Poly-3 Adjusted Rate	8.0%	8.2%	18.2%	16.3%
First Incidence (days)	316T	186	101	247
Poly-3 Test	0.156	0.687	0.274	0.324

Table 9 from GMM 16. Historical Incidence 3/102.

Selected neoplasms in female Heterozygous F1 p53(+/-) mice: High dose comparison

Lesion	*AZT Dose Groups (mg/kg/day)*					
	Vehicle Control	**AZT-H**	**3TC-H**	**NVP-H**	**AZT/3TC-H**	**AZT/3TC/ NVP-H**
Malignant lymphoma (all organs)				**[Historical incidence 3/102]**		
Overall Rate	2/25 (8.0%)	2/28 (8.0%)	1/25 (4.0%)	**5/25** (20.0%)	**4/24** (16.7%)	**4/25** (16.0%)
Poly-3 Adjusted Rate	8.0%	8.2%	4.2%	21.1%	16.9%	16.3%
First Incidence (days)	316T	277	258	251	102	247
Poly-3 Test	-	0.686	0.518N	0.186	0.307	0.324
Poly-3 Test[a]	0.324	0.336	0.182	0.479N	0.628N	-
Mammary gland: Adenoacanthoma or adenocarcinoma				**[Historical incidence 1/102]**		
Overall Rate	1/25 (4.0%)	1/25 (4.0%)	**4/25** (16.0%)	0/23 (0.0%)	**2/23** (8.7%)	1/25 (4.0%)
Poly-3 Adjusted Rate	4.0%	4.2%	16.6%	0.0%	9.5%	4.2%
First Incidence (days)	319T	313	271	-	280	302
Poly-3 Test	-	0.750	0.161	0.527N	0.440	0.751
Poly-3 Test[a]	0.751	0.760N	0.173N	0.519	0.455N	-

Table 10 from GMM 16. *Significant increase or dose trend. [a]Comparison to AZT/3TC/NVP-H dose group).

Hepatocellular neoplasms in male Heterozygous F1 p53(+/-) mice: Combination dose comparison

Lesion	*AZT Dose Groups (mg/kg/day)*			
	Vehicle Control	AZT/3TC/ NVP-L	AZT/3TC/ NVP-M	AZT/3TC/ NVP-H
Liver Hepatocellular Adenoma				
Overall Rate	1/25 (4.0%)	7/25 (28.0%)	7/23 (30.4%)	9/23 (39.1%)
Poly-3 Adjusted Rate	4.4%	28.3%	30.5%	39.4%
First Incidence (days)	313	315T	318T	316T
Poly-3 Test	**0.006***	**0.030***	**0.021***	**0.003***
Adjusted Poly-3 Test[a]	**0.003***	**0.005***	**0.004***	**0.002***
Liver Hepatocellular Adenoma or Carcinoma				
Overall Rate	1/25 (4.0%)	9/25 (36.0%)	8/23 (34.8%)	10/23 (43.5%)
Poly-3 Adjusted Rate	4.4%	36.4%	34.9%	43.8%
First Incidence (days)	313	315T	318T	316T
Poly-3 Test	**0.004***	**0.006***	**0.009***	**0.001***
Adjusted Poly-3 Test[a]	**<0.001***	**<0.001***	**0.004***	**<0.001***

Table 7 from GMM 16. Historical Incidence 8/100. *Significant increase or dose trend. [a]Adjusted for litter cluster correlation (Appendix G).

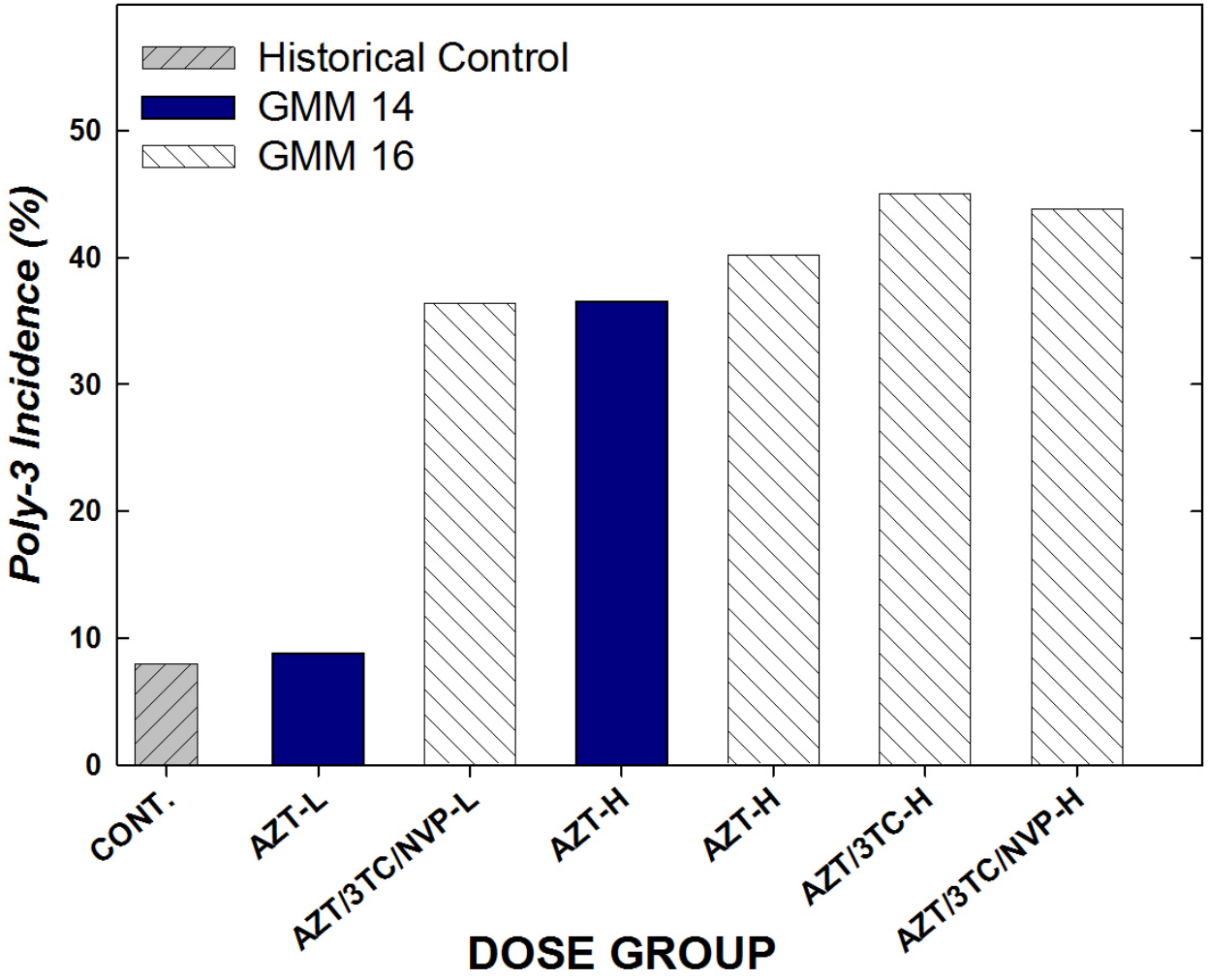
Hepatocellular neoplasms in male Heterozygous F1 p53$^{(+/-)}$ mice: High dose comparison

Lesion	*AZT Dose Groups (mg/kg/day)*					
	Vehicle Control	**AZT-H**	**3TC-H**	**NVP-H**	**AZT/3TC-H**	**AZT/3TC/ NVP-H**
Liver Hepatocellular Adenoma			**[Historical Incidence 8/100]**			
Overall Rate	1/25 (4.0%)	8/23 (34.8%)	3/25 (12.0%)	2/25 (8.0%)	9/25 (36.0%)	9/23 (39.1%)
Poly-3 Adjusted Rate	4.4%	42.2%	12.7%	8.9%	40.5%	39.4%
First Incidence (days)	313	316T	318T	322T	316T	316T
Poly-3 Test	-	**0.004***	0.313	0.493	**0.003***	**0.003***
Poly-3 Test[a]	**0.003***	0.601N	**0.036***	**0.016***	0.589N	-
Liver Hepatocellular Adenoma or Carcinoma			**[Historical Incidence 8/100]**			
Overall Rate	1/25 (4.0%)	8/23 (34.8%)	3/25 (12.0%)	2/25 (8.0%)	10/23 (40.0%)	10/23 (43.5%)
Poly-3 Adjusted Rate	4.4%	40.2%	12.7%	8.9%	45.0%	43.8%
First Incidence (days)	313	316T	318T	322T	316T	316T
Poly-3 Test	-	**0.004***	0.313	0.493	**<0.001***	**0.001***
Poly-3 Test[a]	**0.001***	0.529	**0.017***	**0.007***	0.585N	-

Table 8 from GMM 16. *Significant increase or dose trend.

[a]Comparison to AZT/3TC/NVP-H dose group, N = > AZT/3TC/NVP-H value).

Hepatocellular Carcinoma & Adenoma in Males: GMM14 & GMM 16 Comparison



GMM 16 Significant Observations

- **Significant increase in hepatocellular neoplasms in the male AZT/3TC/NVP-L dose group which correspond to 80/40/80 mg AZT/kg/day (b.i.d.).**
- **Toxicokinetic studies (Appendix I) suggest that a dose of 80/40/80 mg AZT/kg/day results in blood AZT concentrations that are moderately greater than human exposure levels, whereas higher doses produce blood levels that are considerably greater.**
- **No evidence of lesion-clustering within littermates (Appendix G).**
- **NVP exposure to PND28 did not increase incidence of hepatocellular neoplasms at 45 weeks.**

Conclusions – GMM 16 - Females

- **Under the conditions of this gavage study, there was *equivocal evidence* *of carcinogenic activity* of NVP alone, AZT in combination with 3TC, and AZT in combination with 3TC and NVP in female heterozygous F1 $p53^{+/-}$ mice based on the occurrence of malignant lymphoma.**
- **There was *equivocal evidence* *of carcinogenic activity* of 3TC alone in female heterozygous F1 $p53^{+/-}$ mice based on the occurrence of mammary gland adenoacanthoma or adenocarcinoma (combined).**
- **There was *no evidence* *of carcinogenic activity* of AZT alone in female heterozygous F1 $p53^{+/-}$ mice administered 240 mg/kg.**

Conclusions – GMM 16 - Males

- **Under the conditions of this gavage study, there was *clear evidence of carcinogenic activity* of AZT alone in male heterozygous F1 p53$^{+/-}$ mice based on increased incidences of hepatocellular adenoma.**
- **There was *clear evidence of carcinogenic activity* of AZT in combination with 3TC, and AZT in combination with 3TC and NVP in male heterozygous F1 p53$^{+/-}$ mice based on increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined).**
- **There was *no evidence of carcinogenic activity* of 3TC alone in male heterozygous F1 p53$^{+/-}$ mice administered 150 mg/kg.**
- **There was *no evidence of carcinogenic activity* of NVP alone in male heterozygous F1 p53$^{+/-}$ mice administered 168 mg/kg.**