



Chemotherapy treatment in pediatric AML patients leads to a relative increase of colonization with potentially pathogenic bacteria in the gut, despite antibiotic prophylaxis

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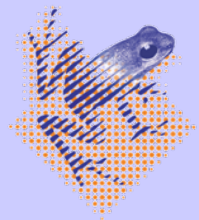
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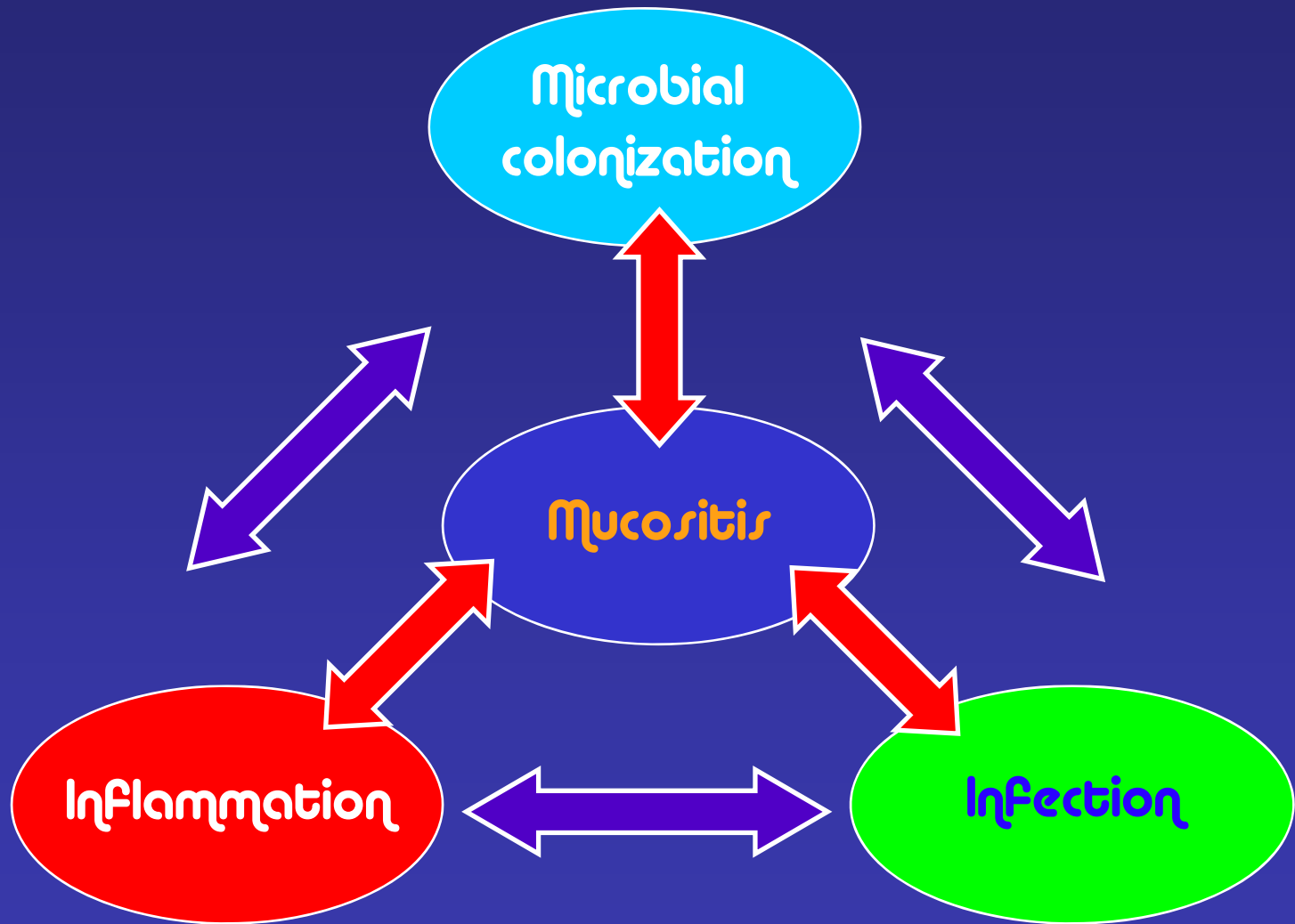
Pediatric oncology

- Intensive chemotherapy treatment has led to an increase in survival in paediatric oncology
- Intensive treatment is associated with
 - Inflammatory complications, such as mucositis
 - Infectious complications, e.g. viridans streptococci
- Patients with acute myeloid leukemia (AML) are most prone for both inflammatory and infectious complications, the reason is unclear.





Inflammatory and infectious complications



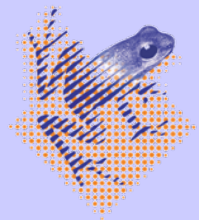


Aim of the study

- Hypothesis: intestinal microbiota plays a role in mucositis and bacterial translocation in AML patients
- Determine the effect of anti-cancer treatment on intestinal microbiota

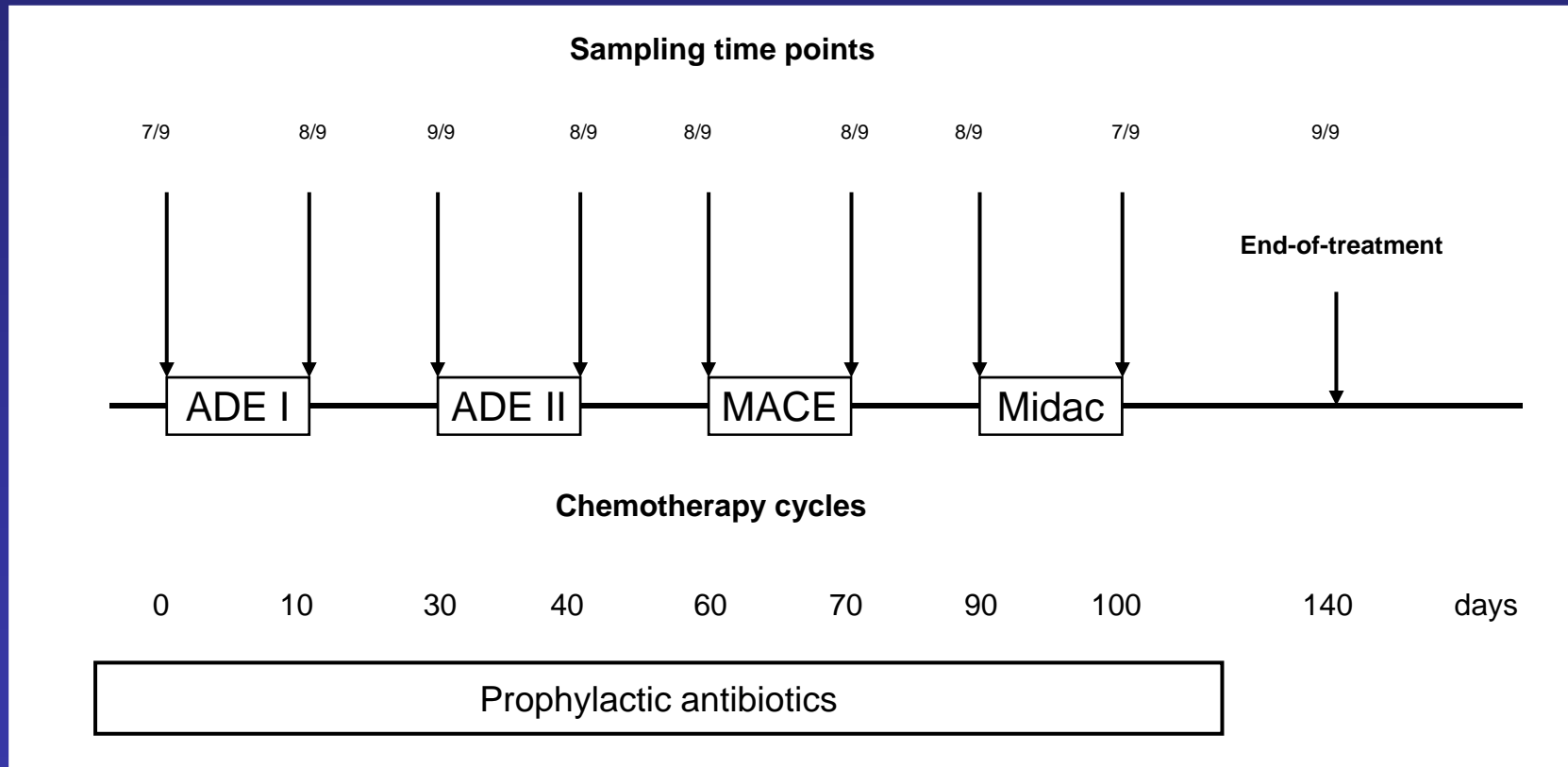
Methods to study microbiota

- Denaturing gradient gel electrophoresis
- Fluorescent *in situ* hybridisation



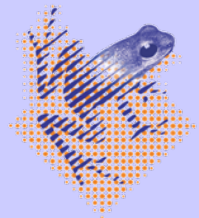


Study design





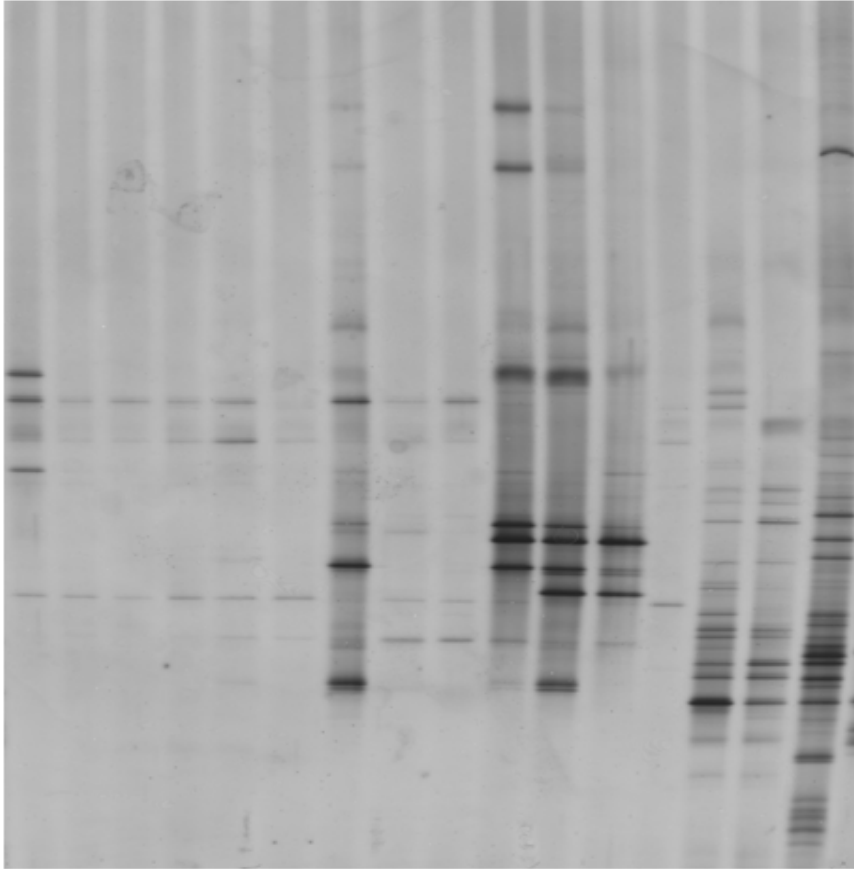
DGGE results



Patient

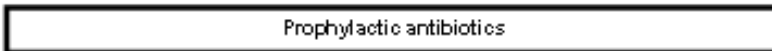
Days

4 9 12 14 16 19 32 36 39 47 58 63 65 100 105 155



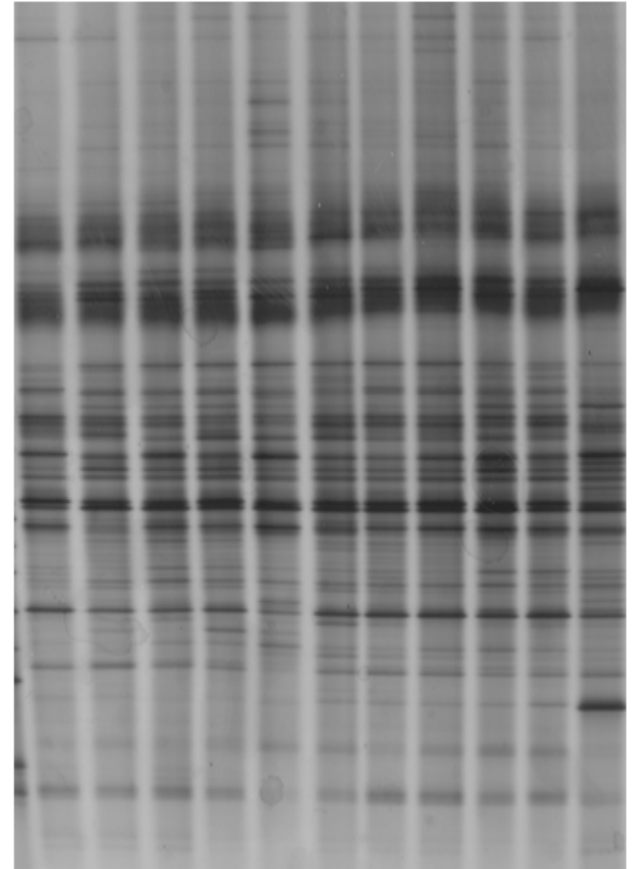
7% 22% 8% 20% 35% 16% 47% 30% 25% 17% 40% 34% 29% 37% 45% 100%

Similarity



Volunteer

1 4 4 7 20 26 28 34 34 39 43

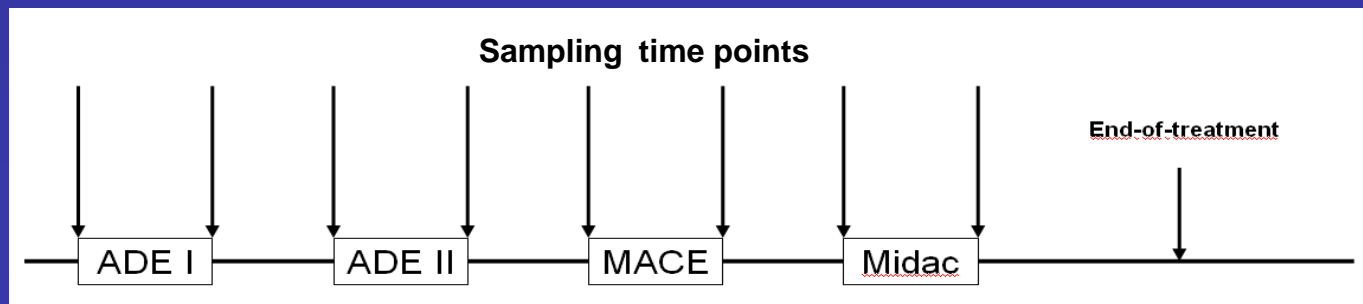


100% 90% 90% 90% 84% 89% 86% 81% 82% 93% 72%



Similarity in DNA fingerprints of microbiota at different time points

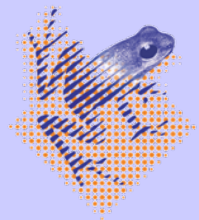
Chemotherapy cycle	Similarity <i>pre-post</i> median (range)	Time point	Similarity with end-of-treatment median (range)
ADE I	16.8 (2.4–82.3)	Pre1	34.7 (4.1–42.0)
		Post1	16.6 (2.4–31.3)
ADE II	51.5 (8.8–72.5)	Pre2	24.9 (3.5–57.4)
		Post2	21.0 (0.0–32.3)
MACE	31.9 (22.7–70.0)	Pre3	20.1 (4.7–59.0)
		Post3	27.6 (10.3–47.1)
MIDAC	64.7 (20.2–86.1)	Pre4	31.6 (15.7–44.3)
		Post4	36.5 (10.6–69.1)
End-of-treatment			100

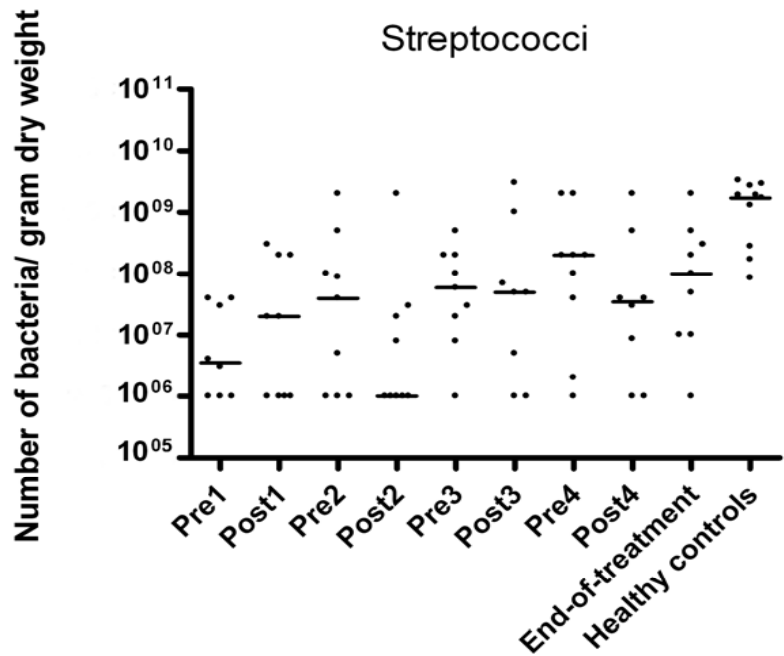
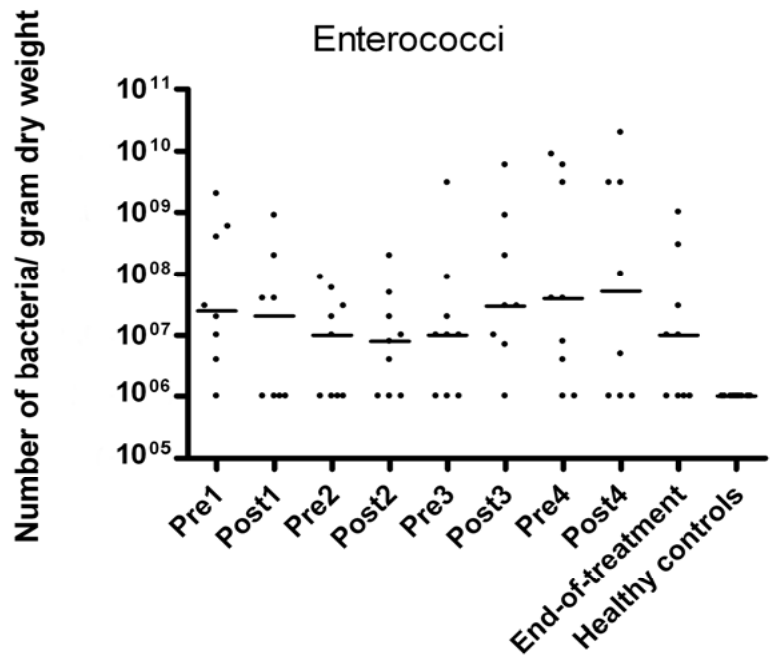
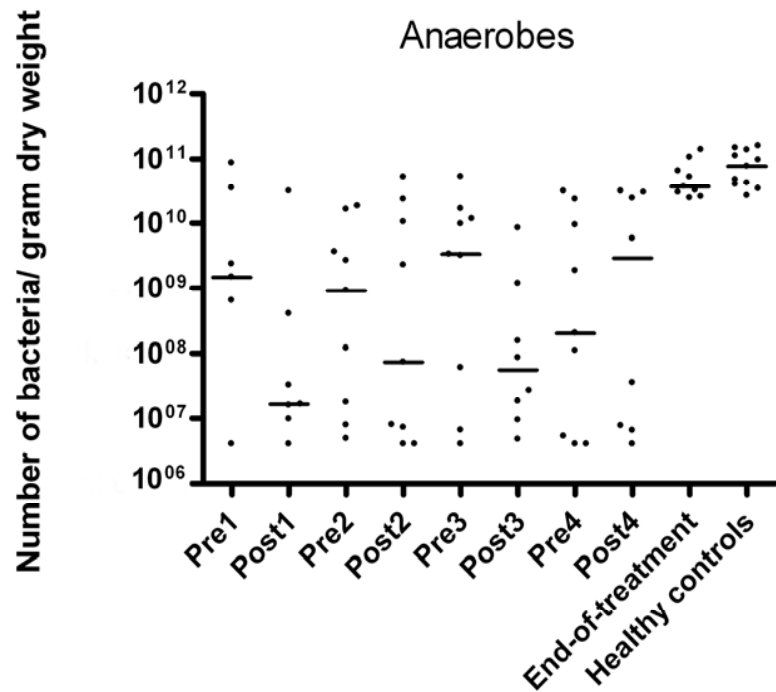
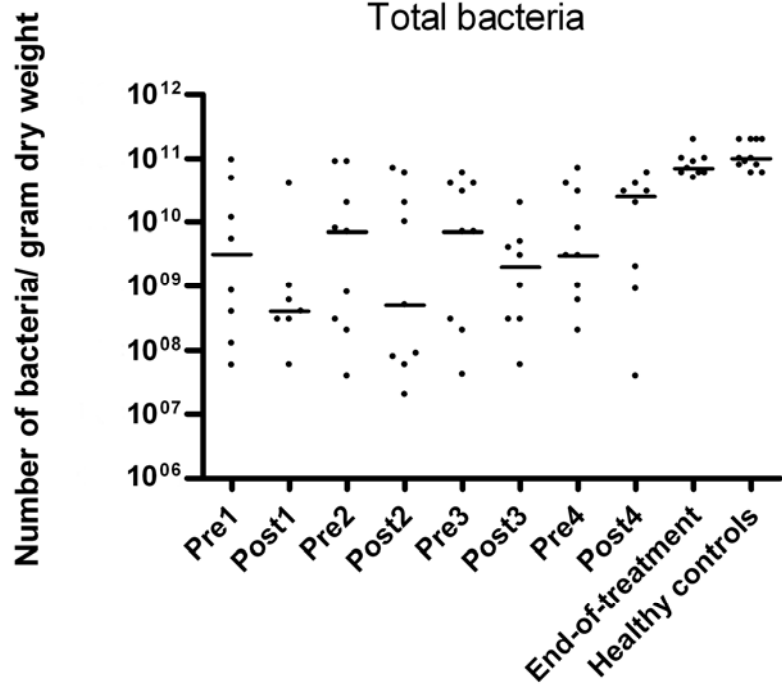




First conclusions

- AML treatment has a large impact on intestinal microbiota
- AML treatment diminishes intestinal microbial diversity
- The effect of treatment varies between individuals







FISH conclusions

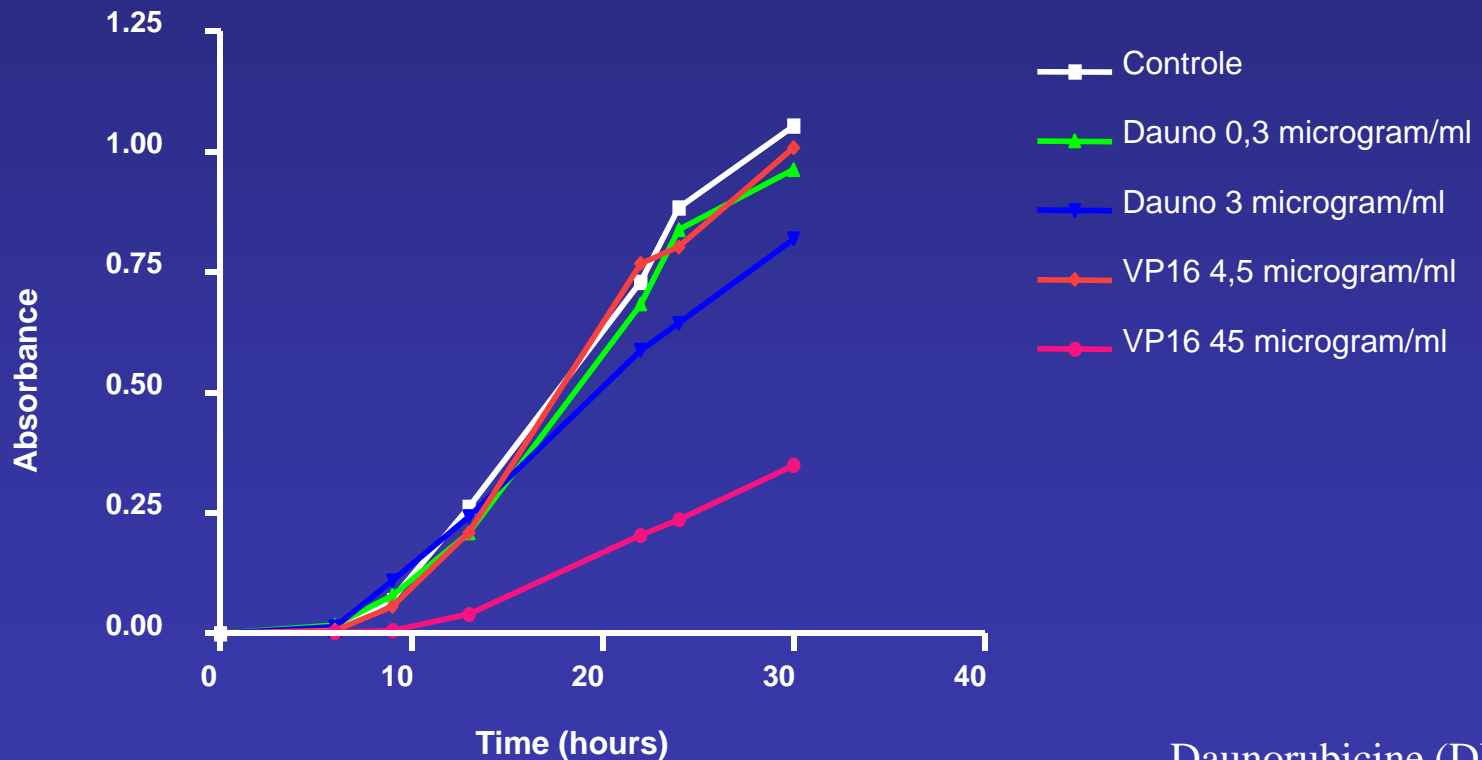
- AML treatment is associated with an up to 10,000 fold decrease in anaerobic bacteria
- Non-strict anaerobes are affected less (streptococci) or show even an increase (enterococci)



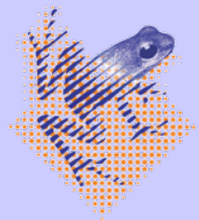


In vitro effects of chemotherapeutics on bacterial growth (1)

B. vulgatus



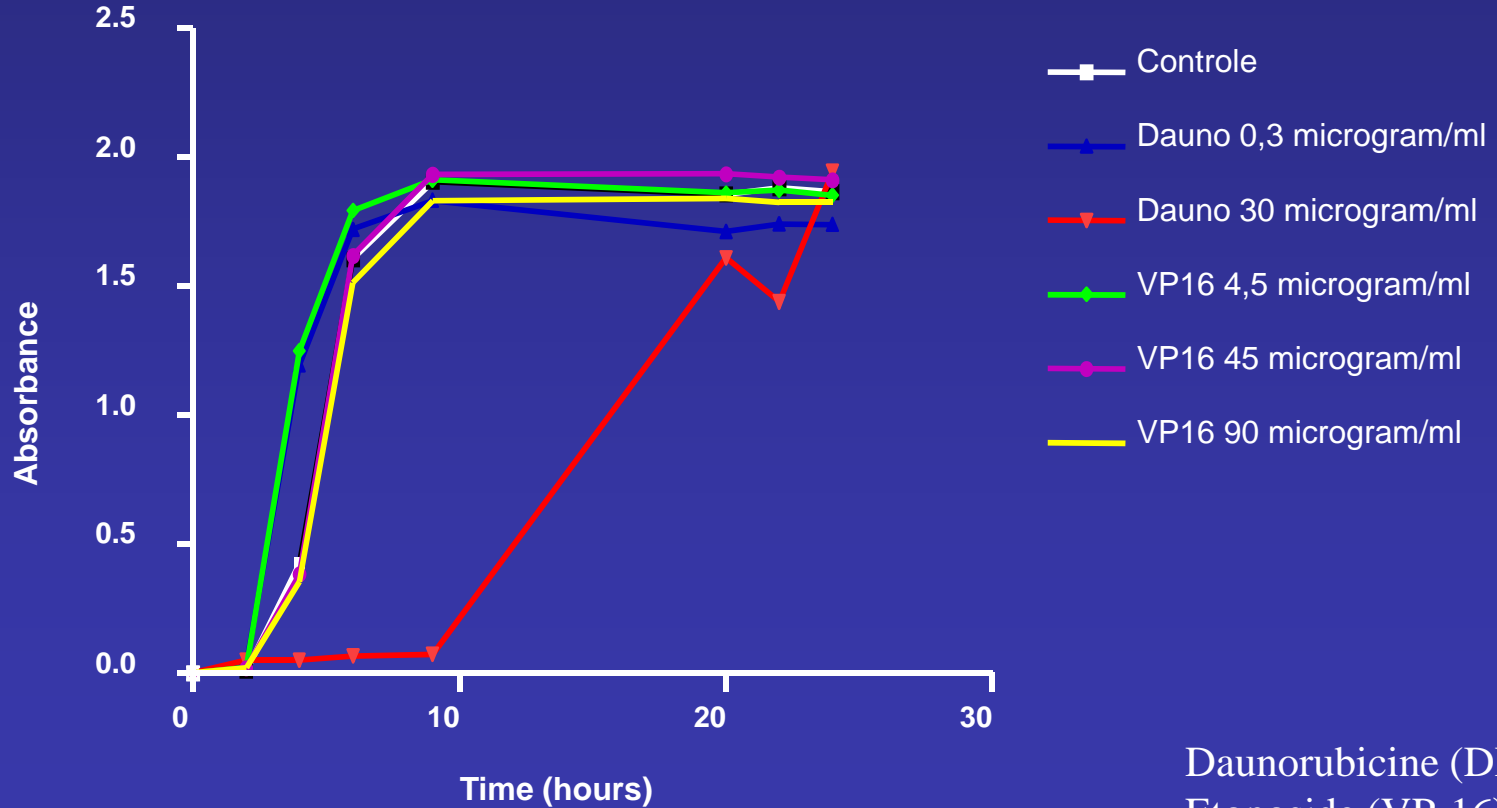
Daunorubicine (DNR),
Etoposide (VP-16)





In vitro effects of chemotherapeutics on bacterial growth (2)

E faecium



Daunorubicine (DNR),
Etoposide (VP-16)





In vitro sensitivity of bacterial strains for Daunorubicine (DNR), Etoposide (VP-16)

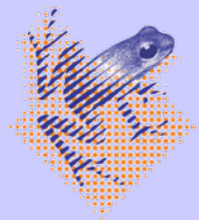
	DNR 0.3 ug/ml	DNR 3 ug/ml	VP-16 4.5 ug/ml	VP-16 45 ug/ml
Aerobic bacteria				
<i>Streptococcus mitis</i>	0.07	0.03	0.04	0.03
<i>Enterococcus faecalis</i>	0.98	0.95	0.99	0.03
<i>Escherichia coli</i>	ND	1.05	ND	0.93
Anaerobic bacteria				
<i>Bacteroides distasonis</i>	0.85	0.75	0.66	0.36
<i>Clostridium difficile</i>	0.96	0.90	0.03	0.03
<i>Clostridium ramosum</i>	0.76	0.52	0.90	0.06
<i>Lactobacillus acidophilus</i>	0.92	0.94	0.00	0.01
<i>Bifidobacterium animalis</i>	0.97	0.69	0.66	0.35





Chemotherapeutics: conclusions

- *In vitro* chemotherapeutics such as Daunorubicin and Etoposide have strong and dose-dependent effects on bacterial growth





Take home messages

- Anti-cancer treatment has large effects on intestinal anaerobic microbiota
- Non-strict anaerobes are *in vivo* less affected by treatment
- Chemotherapeutics have strong inhibitory effects on bacterial growth *in vitro*





Acknowledgements



SKOG

- UMCG

- Department of Paediatric Oncology

MJ van Vliet

Dr ESJM de Bont

Dr WJE Tissing

Prof Dr WA Kamps

- Center for Liver, Intestines and Metabolic Diseases and
Department of Paediatrics

Dr Ir F Stellaard

Dr EHHM Rings

Dr HA Koetse

- Department of Medical Microbiology

Dr HJM Harmsen

Prof Dr JE Degener

Dr NEL Meessen

- Radboud UMCN

- Department of Hematology

Dr NMA Blijlevens

Dr JP Donnelly

