

Gene  
Therapy

Rescue of  
immunodeficiency in  
patients with Bloom  
Syndrome through  
AAV9

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Bloom Syndrome is a rare autosomal recessive disorder of chromosomal instability

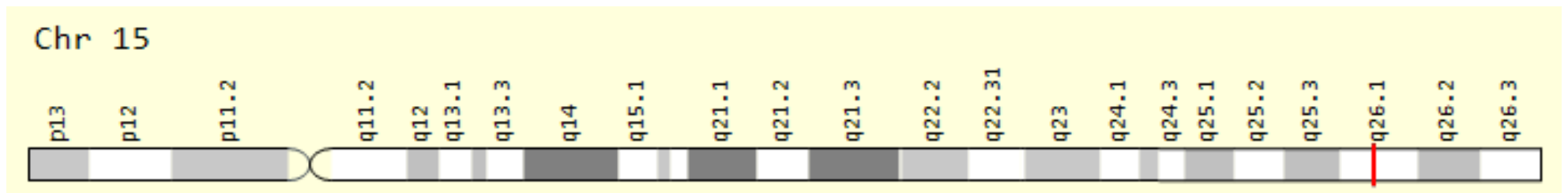
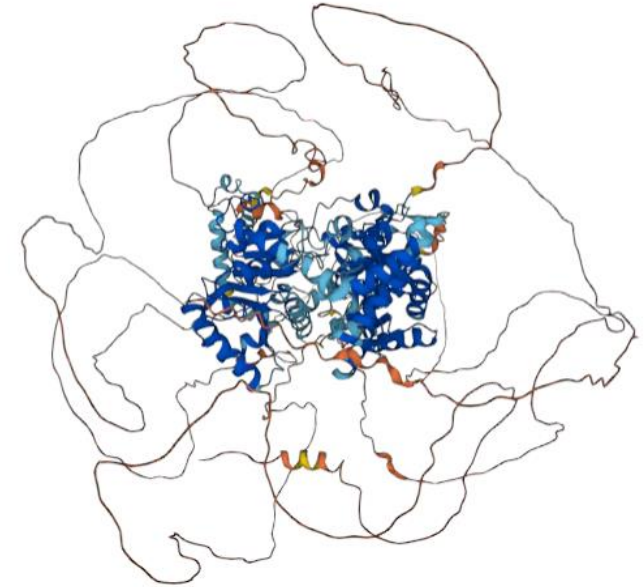
Symptoms:

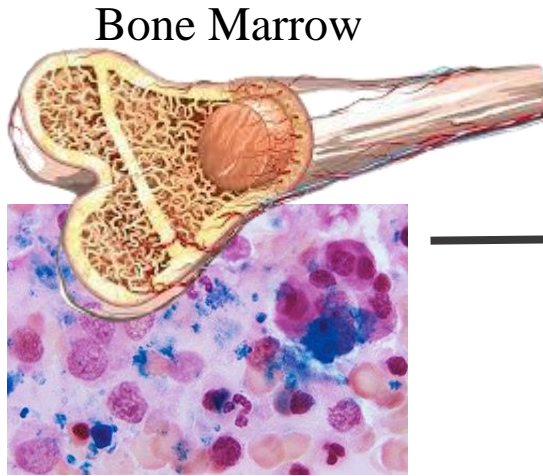
- Immunodeficiency
- Chromosomal instability and early epigenetic aging
- Extreme sun sensitivity
- Recurrent infections
- Predisposition for malignancies and diabetes
- Developmental delays and intellectual differences

Cause:

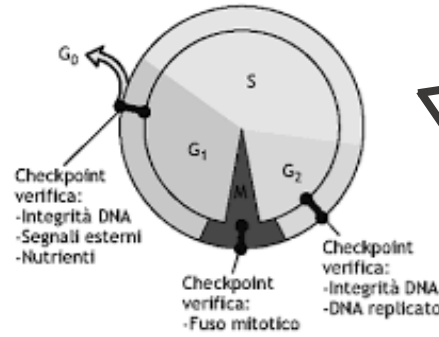
- Mutation of BLM gene located at 15q26.1

The *BLM* gene encodes a RecQ helicase, RECQL3, that unwinds a variety of DNA substrates; therefore is involved in several pathways contributing to the maintenance of genome stability

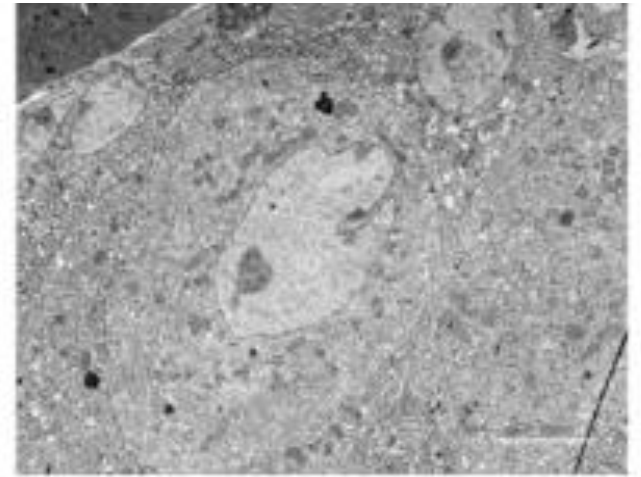




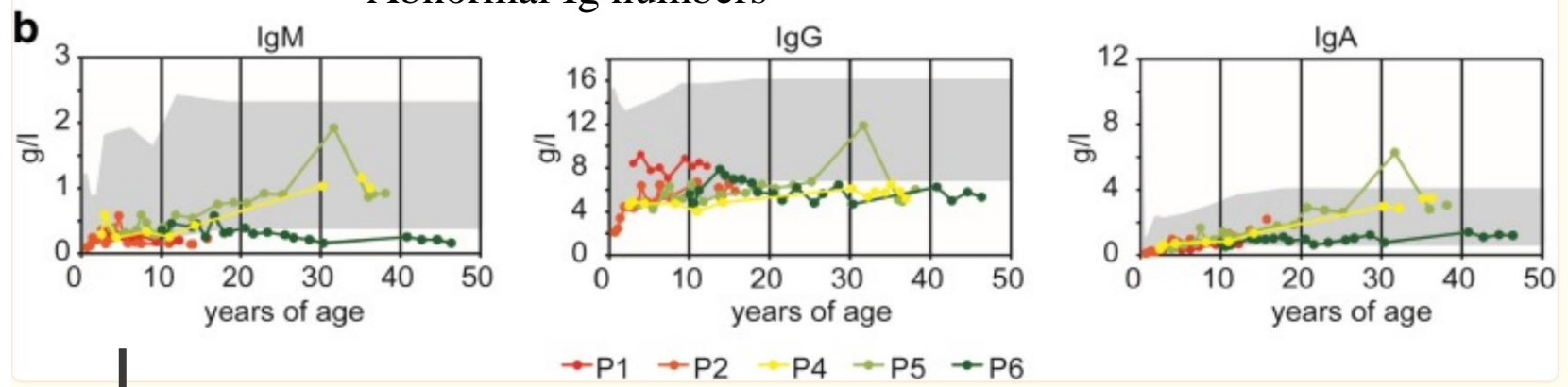
Dysregulated cell cycle



Abnormal B cells' morphology



Abnormal Ig numbers



- Decreased quality of life
- Increase mortality rate at younger age

Our aim is to:

- Increase the quality of life
- Decrease mortality rate and increase life duration

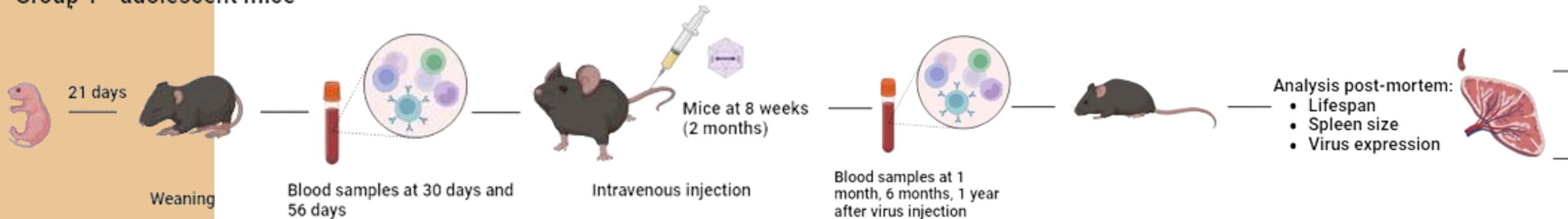


Aim of the project --> Deliver, through AAV9, the correct BLM gene in the lymphocyte in the bone marrow in order to rescue immunodeficiency

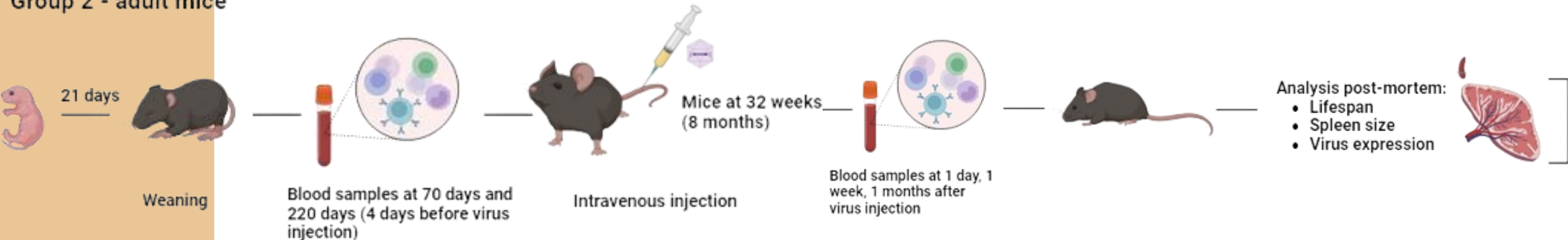


## Experimental design

### Group 1 - adolescent mice



### Group 2 - adult mice

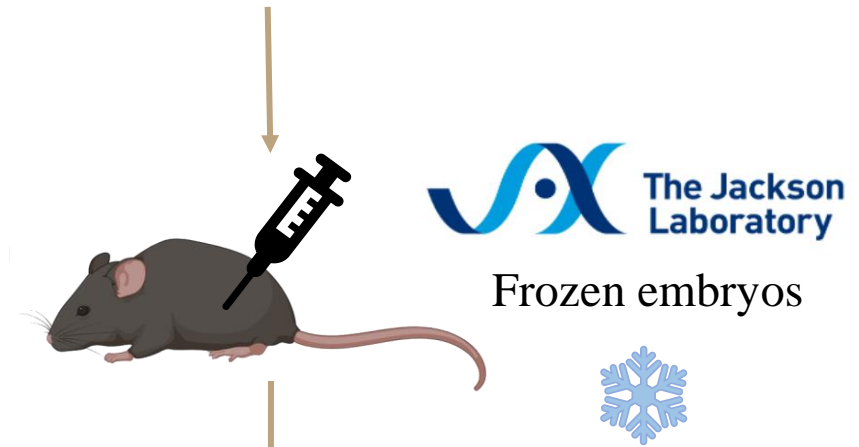
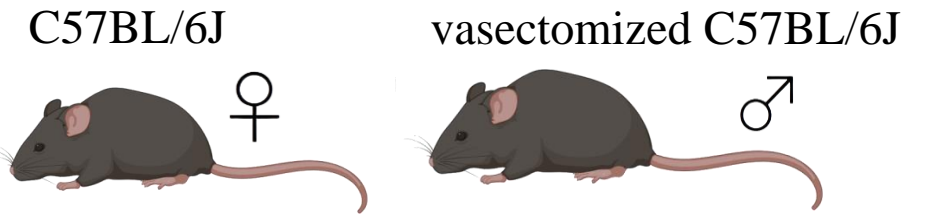


# Mouse model



Phenotypes:

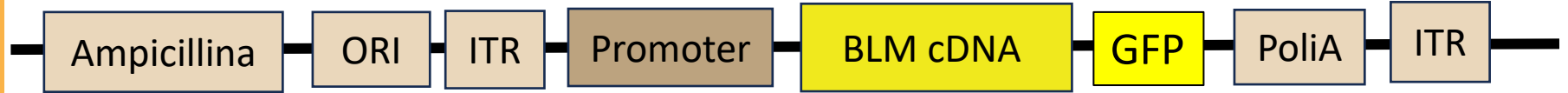
Affected Systems	cn1	cn2
<a href="#">show</a> or <a href="#">hide</a> all annotated terms		
<b>cellular</b>		✓
elevated level of mitotic sister chromatid exchange		✓
<b>hematopoietic system</b>	✓	✓
abnormal B cell morphology		✓
decreased B cell number		✓
small spleen		✓
abnormal B cell physiology		✓
abnormal class switch recombination	✓	
<b>immune system</b>	✓	✓
abnormal B cell morphology		✓
decreased B cell number		✓
small spleen		✓
abnormal B cell physiology		✓
abnormal class switch recombination	✓	



STOCK *Blm*<sup>tm4Ches/J</sup>

# Vector design

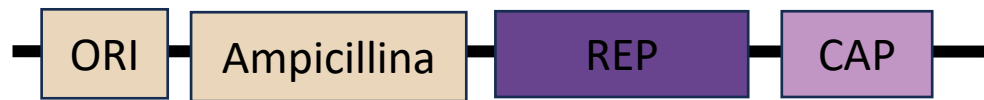
Recombinant AAV9



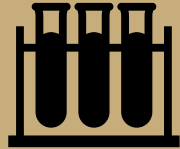
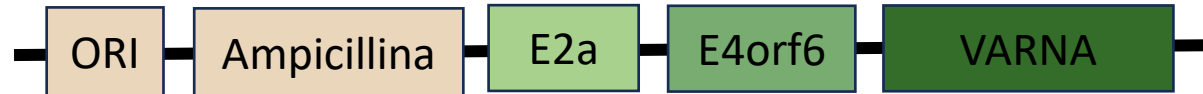
The promoter of the CD19 gene is a target for the B-cell-specific transcription factor BSAP

Z Kozmik<sup>1</sup>, S Wang, P Dörfler, B Adams, M Busslinger

AAV9 Rep-Cap



Helper plasmid



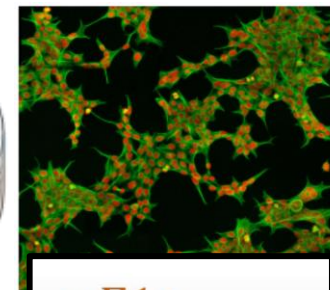
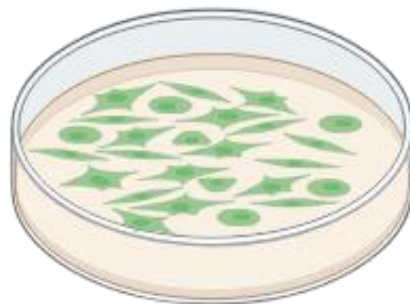
Recombinant AAV9

AAV9 Rep-Cap

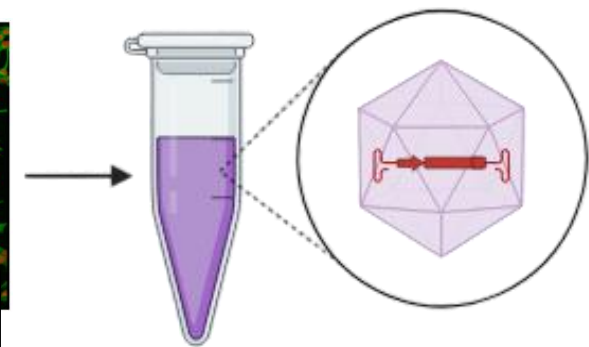
Helper plasmid



HEK293 cells



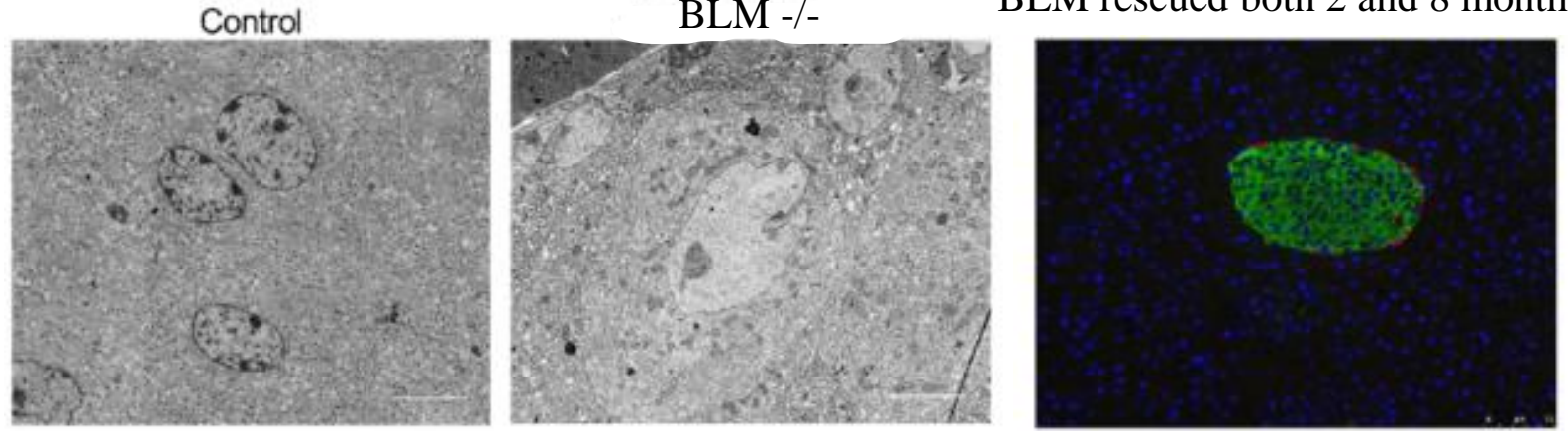
- E1a
- E1b55k



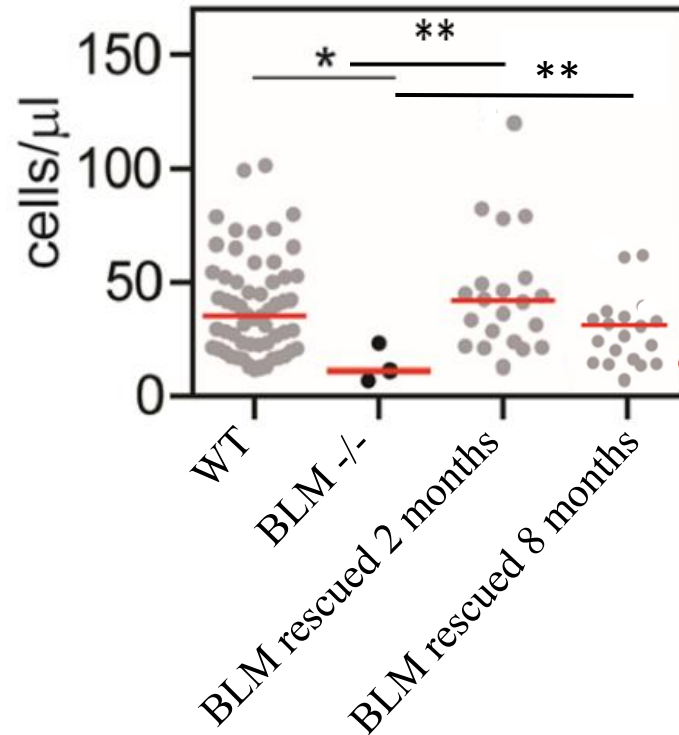
# Expected results

Blood sample

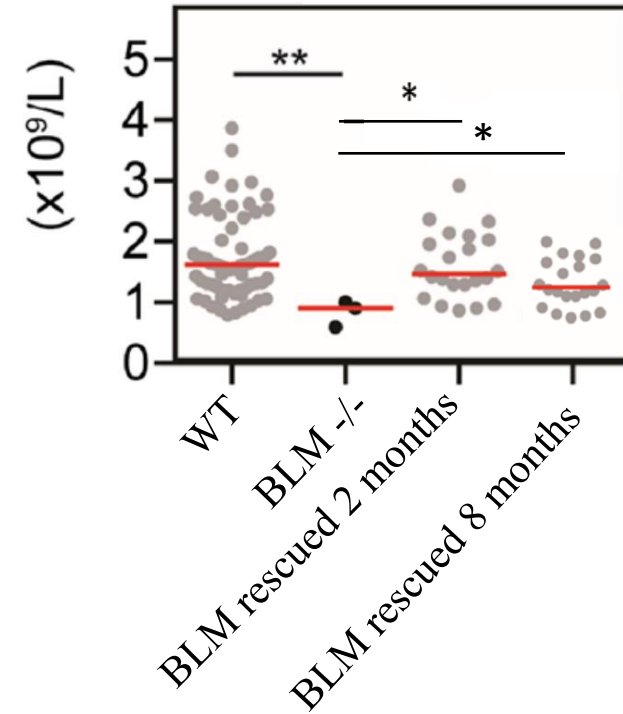
## Lymphocytes B morphology



## B cells number

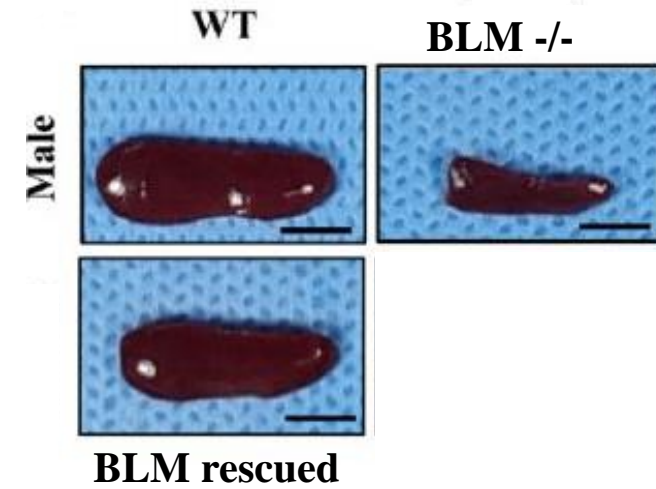
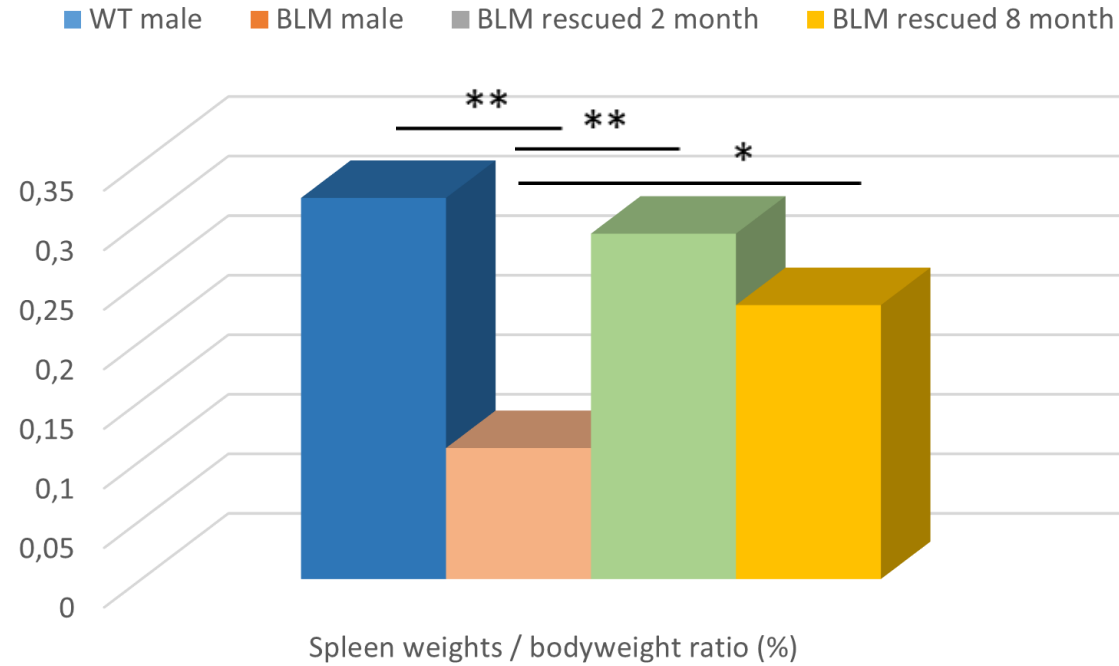


## Immunoglobulins

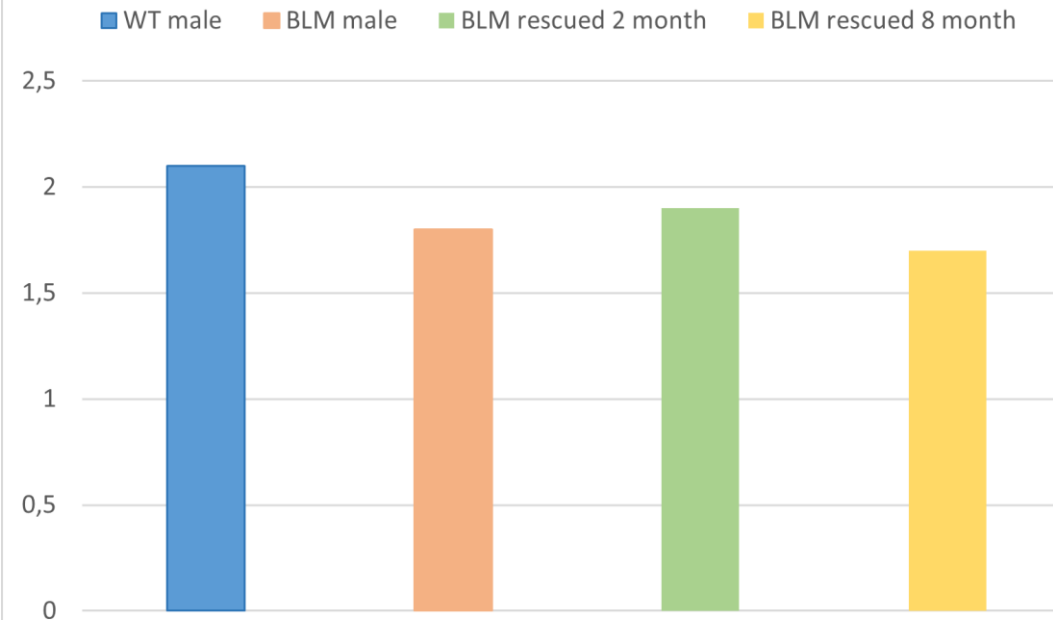


# Expected results

## Spleen size



## Lifespan





# Conclusions



## Blood sample:

Rescue of the numbers of Ig and lymphocyte B. For the latter there was a rescue also for morphology

## Spleen size:

Spleen size returned to normal condition, therefore highlighting a rescue

## Lifespan:

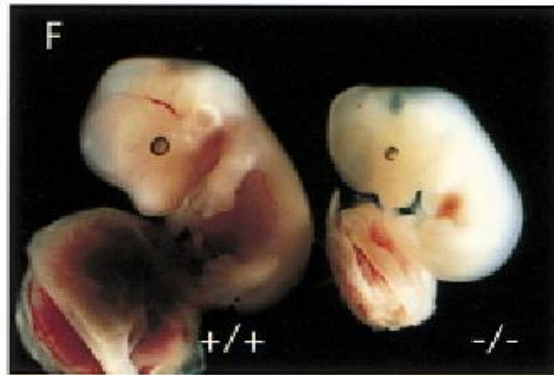
There was no difference in lifespan between all the groups. However, mice, in contrast to humans, do not present a shortened lifespan. Moreover, mice are in sterile conditions, not applicable in human cases

# Future direction

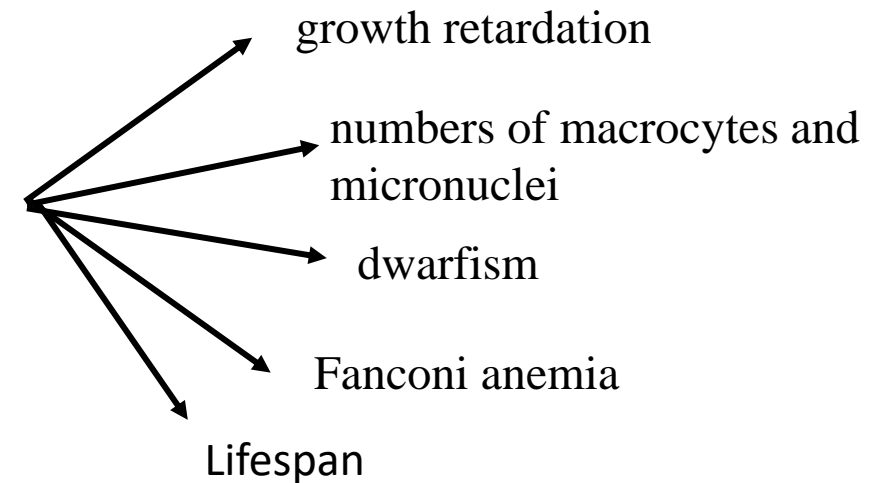
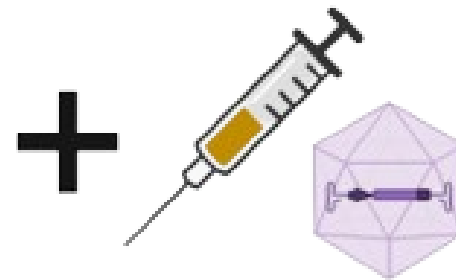
Pre-clinical phase

## Stage-specific apoptosis, developmental delay, and embryonic lethality in mice homozygous for a targeted disruption in the murine Bloom's syndrome gene

N Chester<sup>1</sup>, F Kuo, C Kozak, C D O'Hara, P Leder

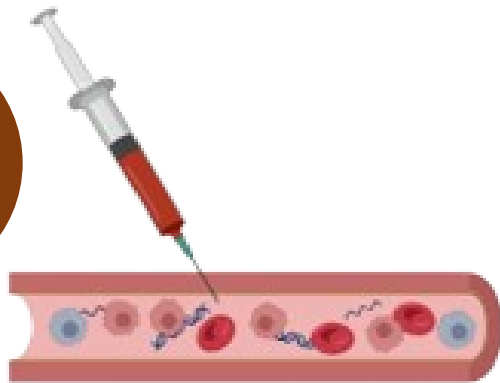
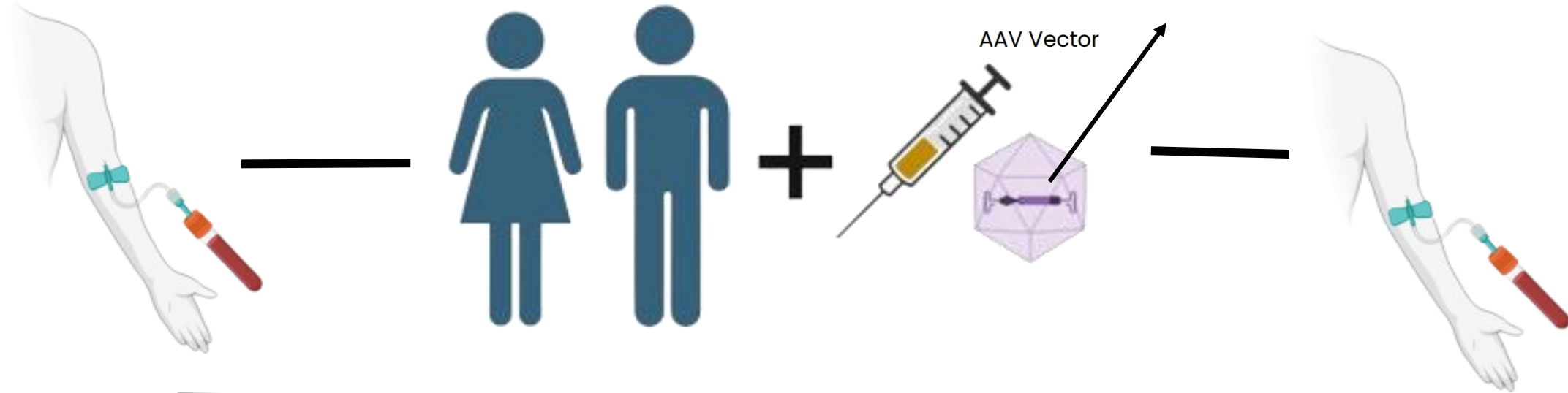


Embryonic Stage



# Future direction

## Clinical phase



B cells number

B cells morphology

B cells physiology

Immunoglobulins

- Frequency of infections
- Lifespan

# Pitfalls and solutions

## Stage-specific apoptosis, developmental delay, and embryonic lethality in mice homozygous for a targeted disruption in the murine Bloom's syndrome gene

N Chester<sup>1</sup>, F Kuo, C Kozak, C D O'Hara, P Leder

Chester et al. demonstrated how homozygosity in mice can be lethal even in the embryonic state. This could represent a complication in the number of experimental subjects.



Ensure that heterozygotes exhibit the same phenotype and use the latter in order to increase the number of experimental subjects

AAV is a stable virus, however the episome is deleted after certain number of cell cycle. (More a cell proliferate, the less the episomes last)



Evaluate whether and when a second injection is necessary





# Budget

What	Cost	
	Each	Units
Frozen embryos	3,968.90 €	x 4 = 15.875,6 €
C57 (Male and Female)	35 €	x 8 female + 4 male = 420 €
Mice Rack ventilated cages	2.500 €	
Mice maintainance (food and sawdust)	9 000 €/year	
Mask for resercher	2.990 €	x3 = 8979 €
Other DPI	2.000 €	
Basic lab equipments (Ex:plastic)	4.000 €	
AAV9	2.300 €	
cDNA	675 € (1 ug)	
GFP	824 €	
HEK cells	595 €	
customs clearance	500 €	
Blood analysis, Physiology evaluation	4.000 €	
immunoistochemetry	1.500 €	
Research team	80 000 €/year for 2 Phd and 1 Post-Doc	
		Tot= 133 168 €



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- <https://www.atcc.org/products/crl-1573>