Deficit di antitripsina e serpinopatie – polimeri patologici e terapia con piccole molecole

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Vice Provost (Health) and Head of

University College London Medical School

Dichiarazione di interessi

Ho un brevetto per le piccole molecole descritte in questa presentazione. Sono stati concessi in licenza a una società statunitense per passare agli studi sull'uomo (spero quest'anno)

Deficit di Z α_1-antitripsina

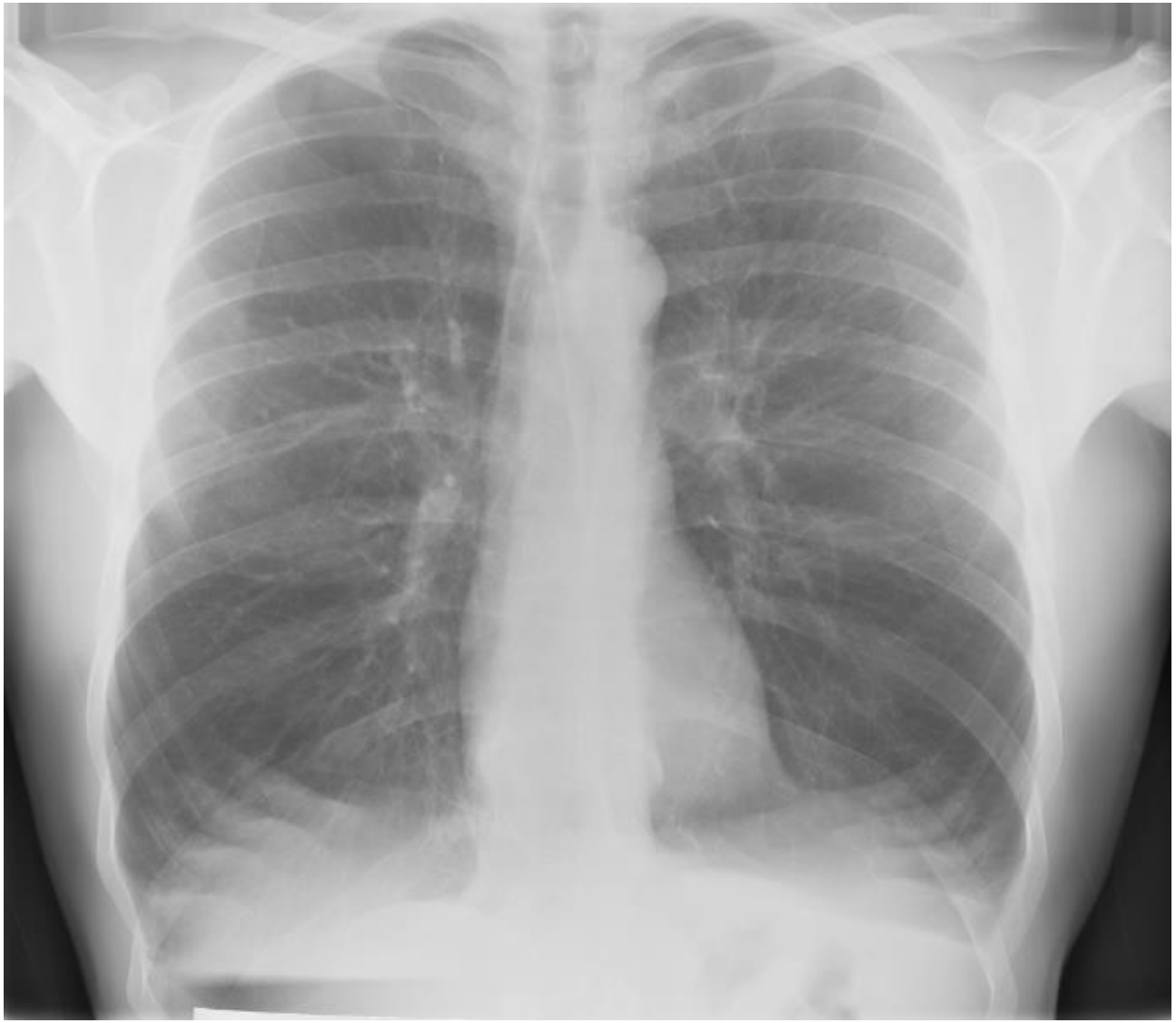
^{342}Glu to Lys

1:1700 North European Caucasians

plasma antitrypsin level 10-15% of normal

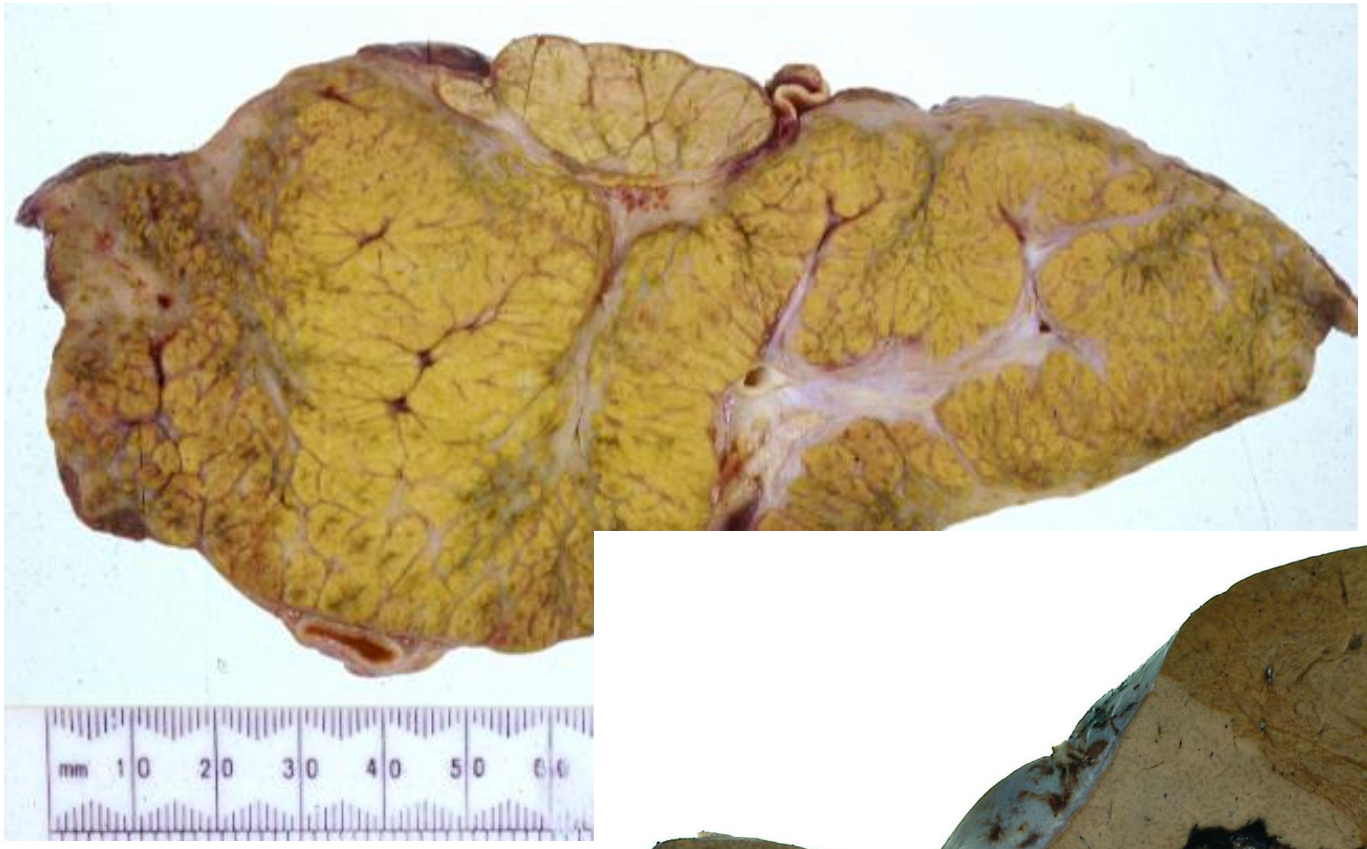
85% protein is retained in the liver

Enfisema con deficit di α_1-antitripsina

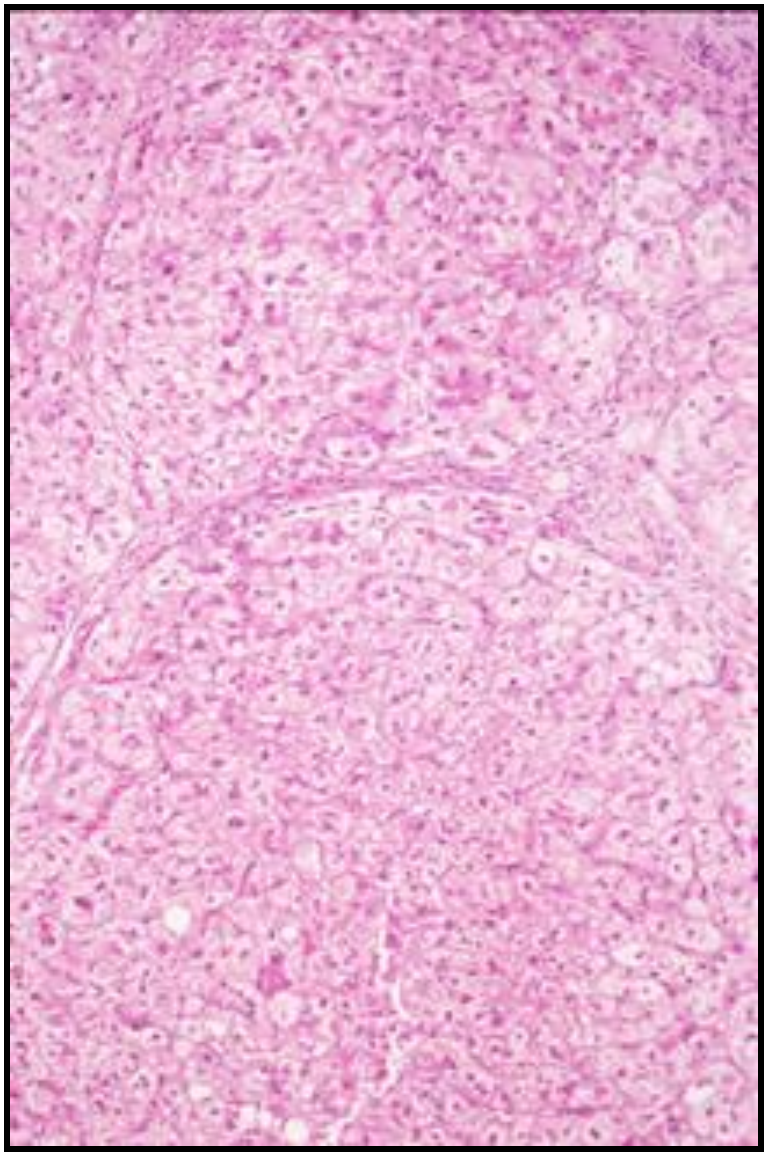


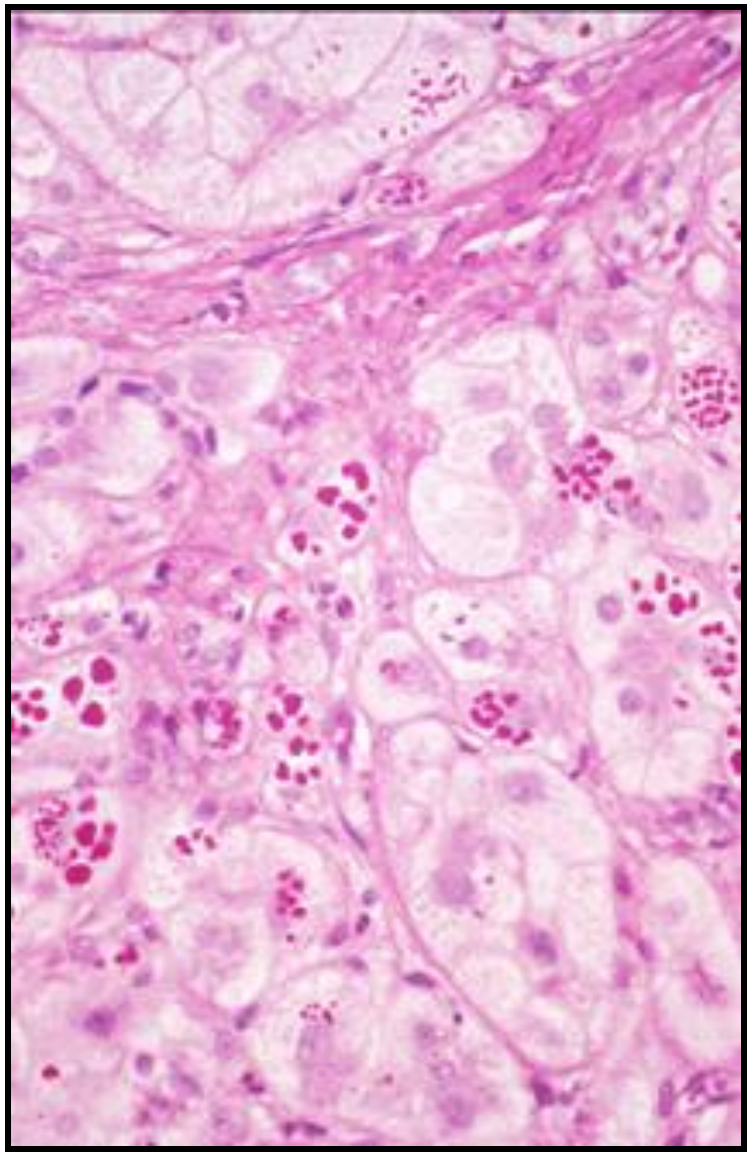
Eriksson, *Acta Med. Scand.* 1965; suppl 432: 1-85

Cirrosi con deficit di α_1-antitripsina

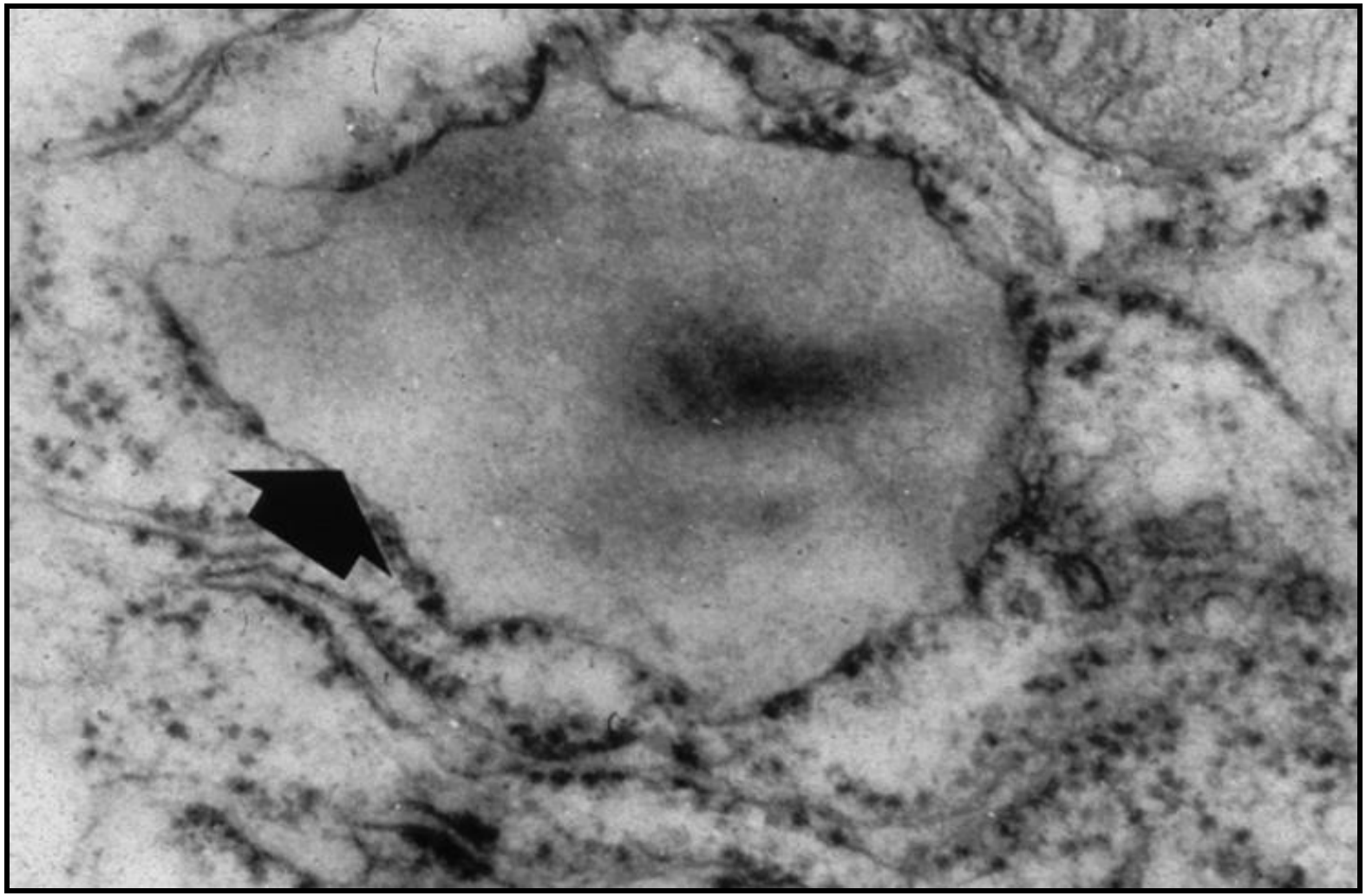


Z α_1-antitripsina forma inclusioni PAS positive

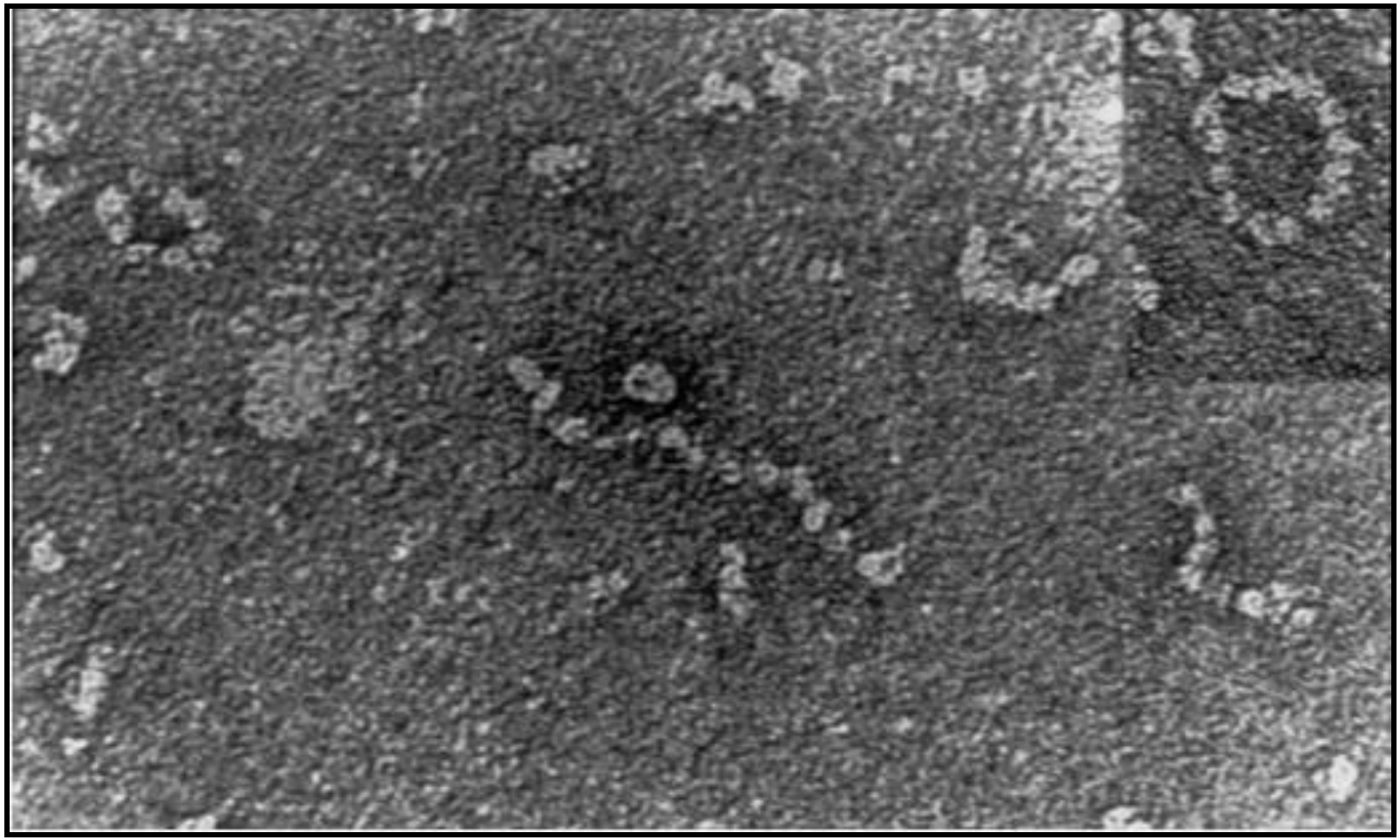




Z α_1-antitripsina viene trattenuta nel ER

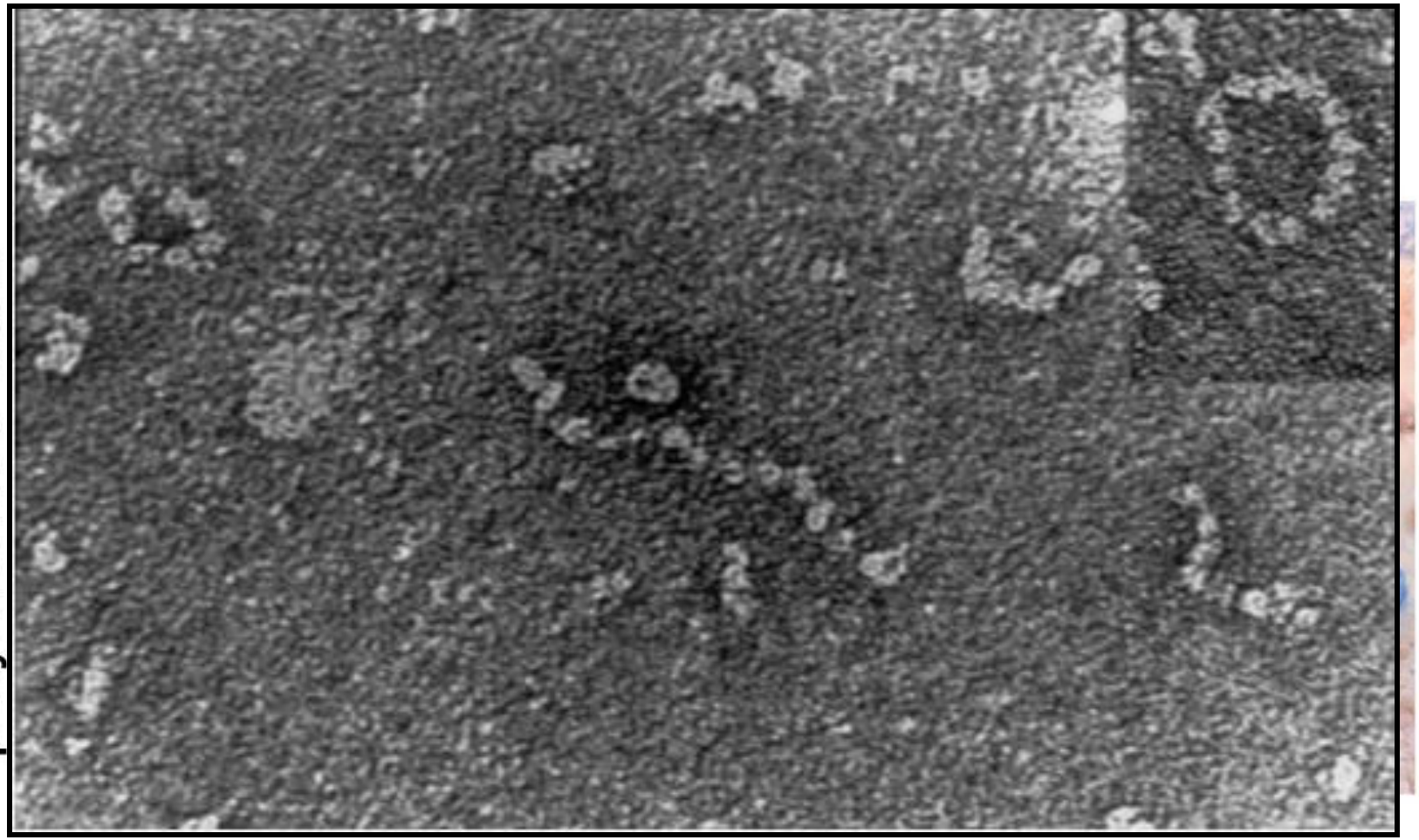


Z α_1-antitripsina forma polimeri *in vivo*



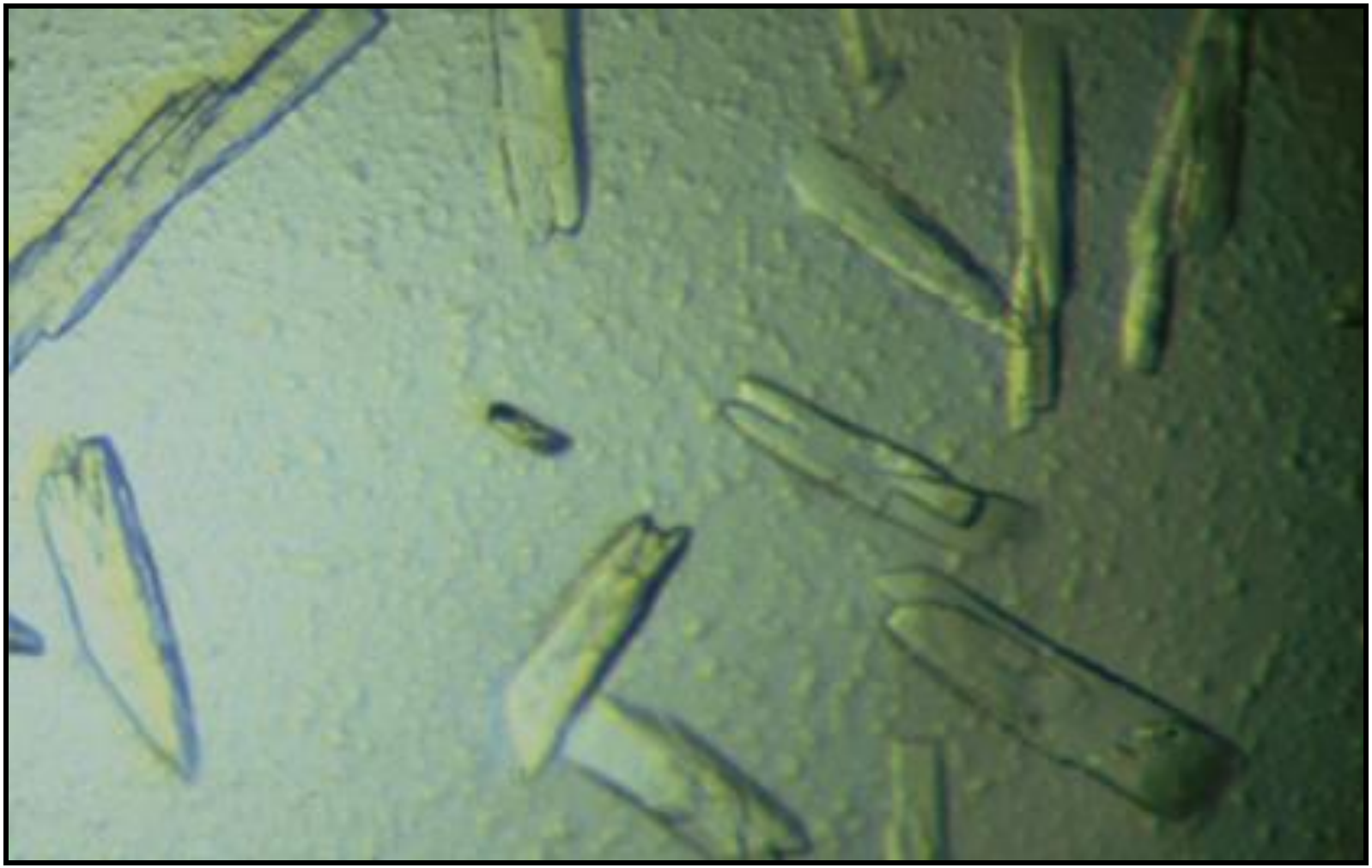
Lomas *et al*, *Nature* 1992; 357: 605-607
Lomas *et al, J.Biol.Chem.* 1993; 268: 15333-15335

L'anticorpo monoclonale anti-polimero colora le inclusioni

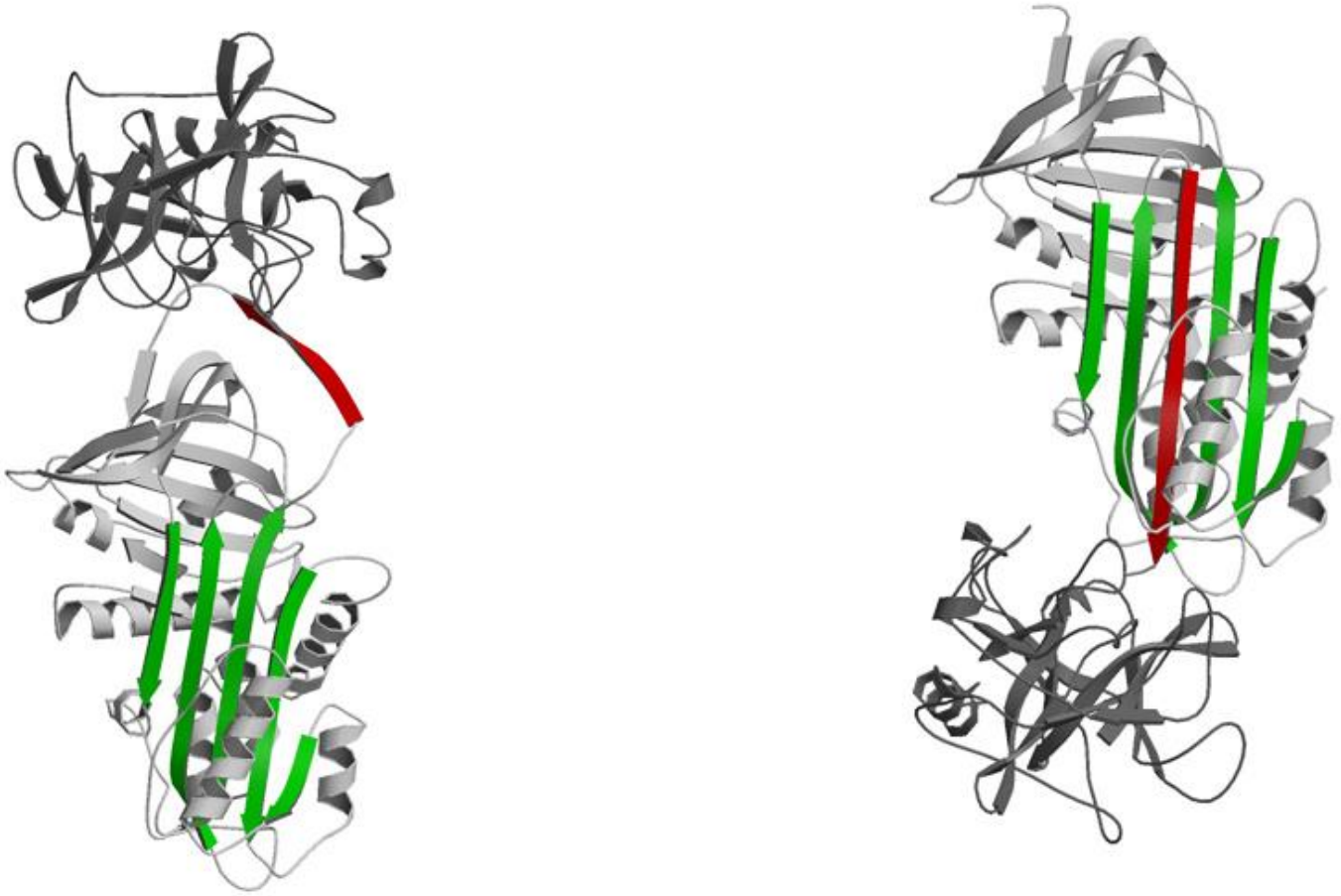


Miranda *et al*, *Hepatology* 2010; 52: 1078-1088

Struttura cristallina dell'α_1- antitripsina

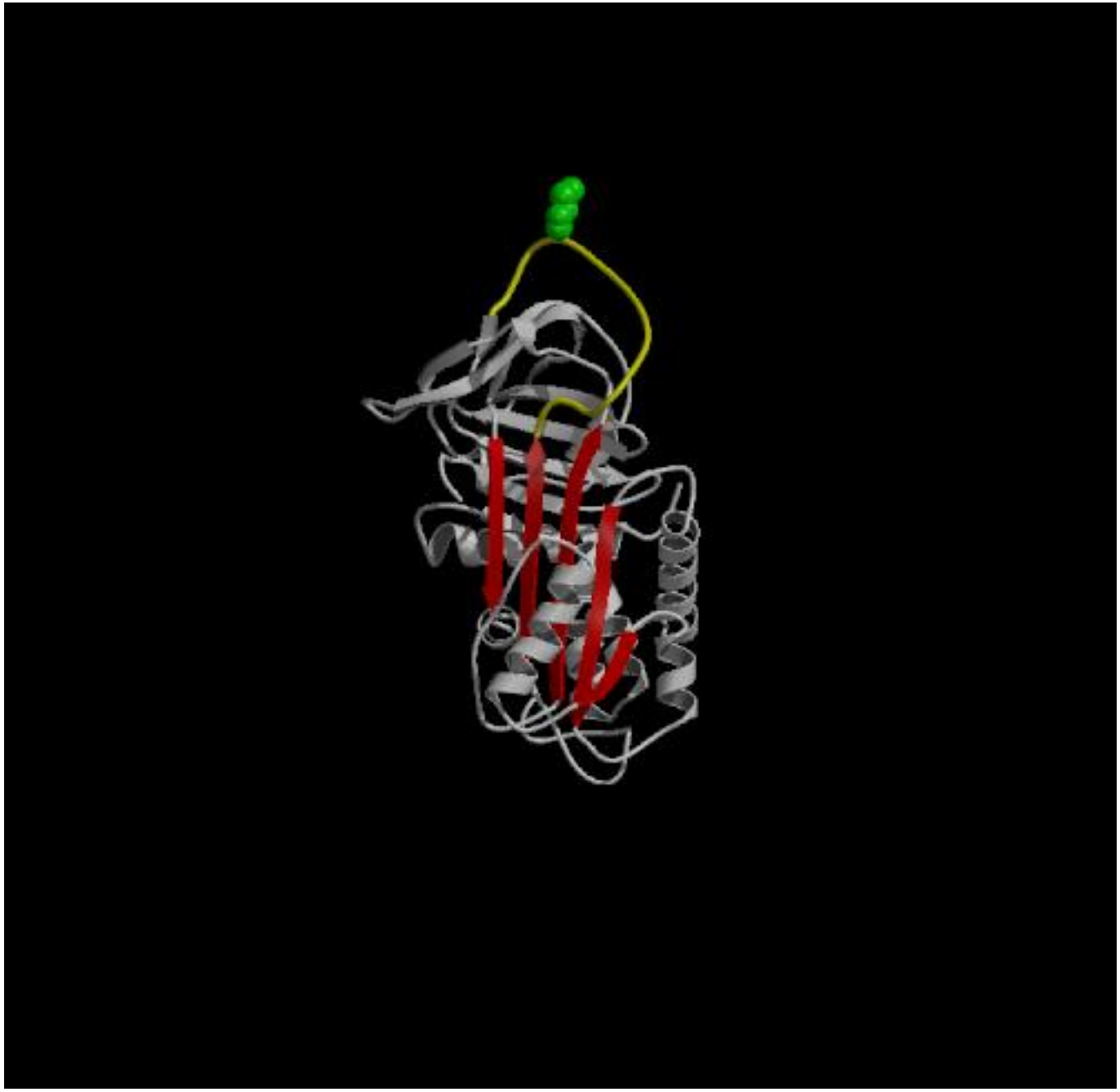


Inibizione di α_1-antitripsina dell'elastasi neutrofila

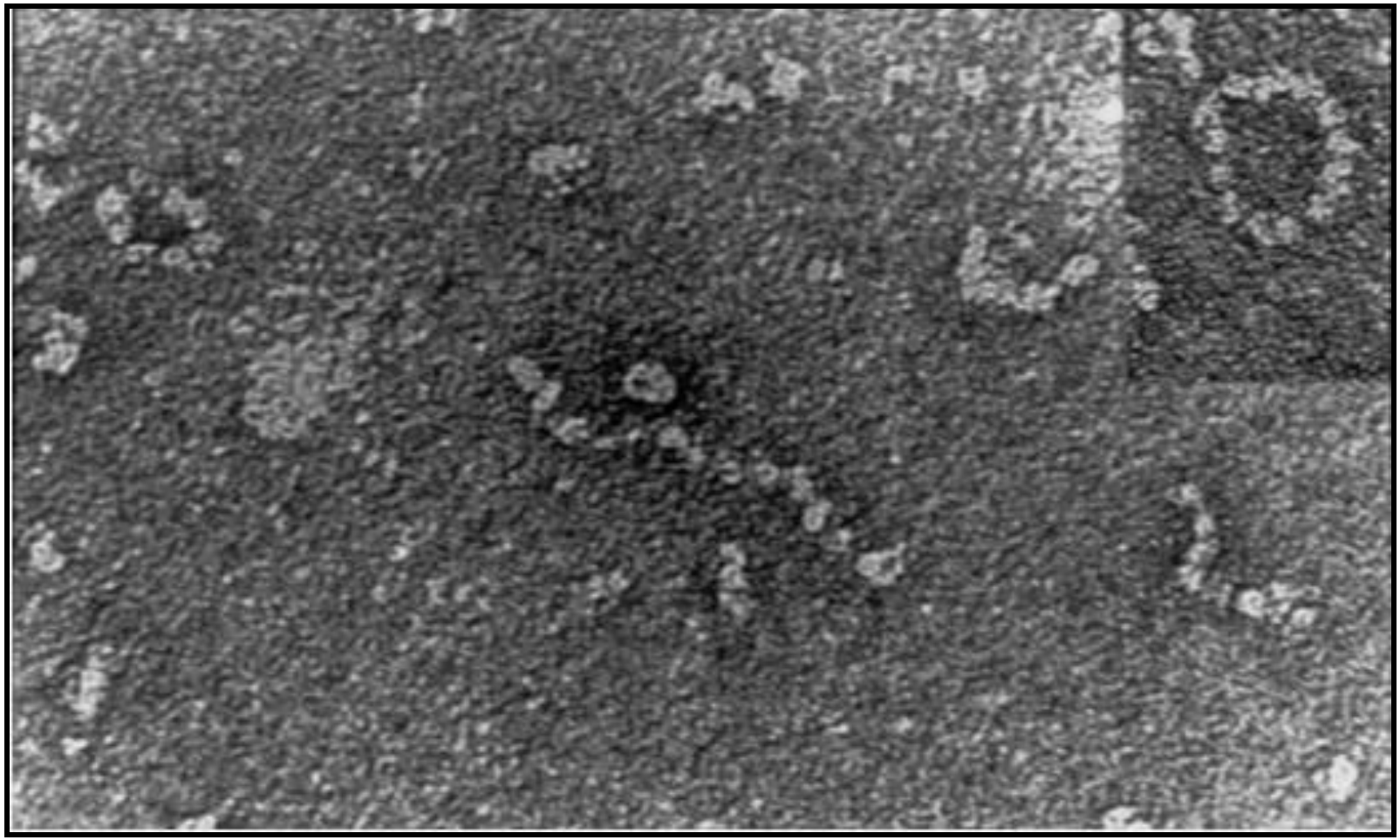


Elliott *et al*, *Nature Struct. Biol.* 1996; 3: 676-681

Huntington *et al*, *Nature* 2000; 407: 923-926

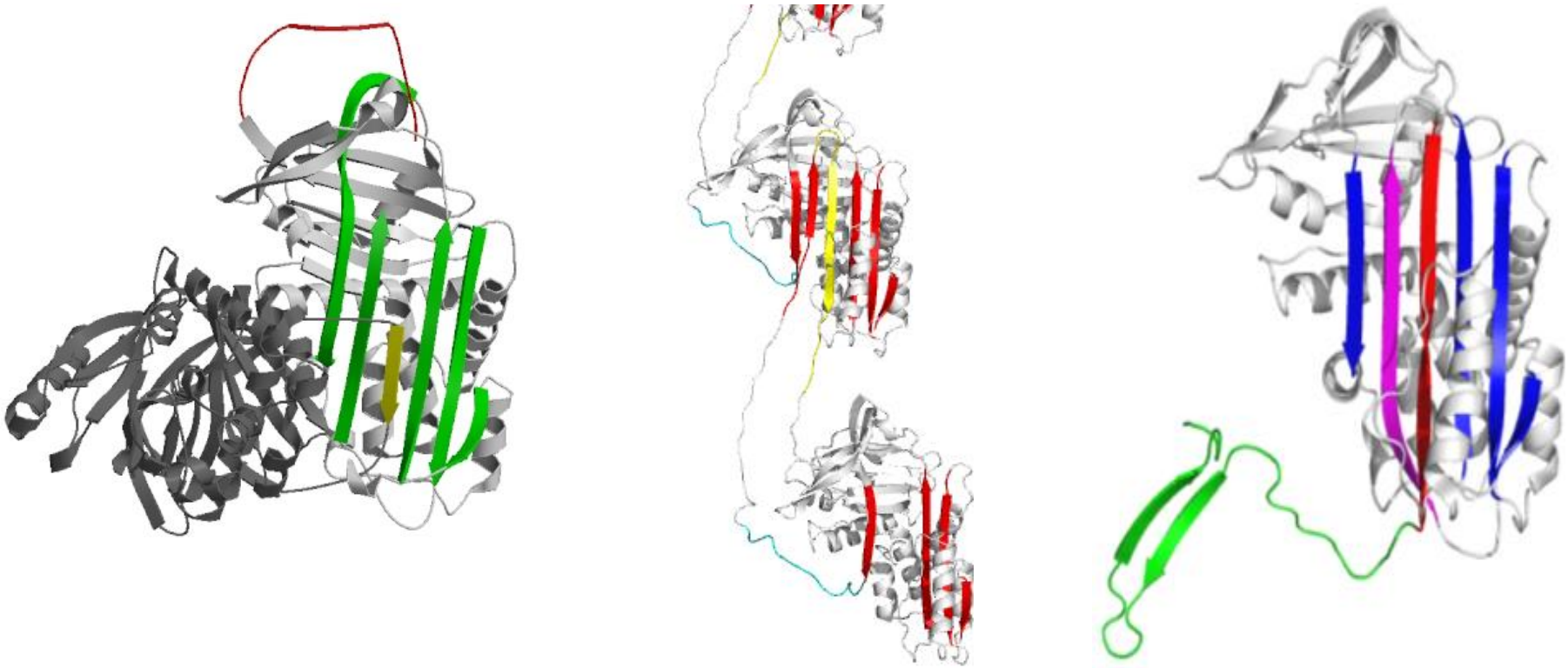


Z α_1-antitripsina forma polimeri *in vivo*



Lomas *et al*, *Nature* 1992; 357: 605-607
Lomas *et al, J.Biol.Chem.* 1993; 268: 15333-15335

Il polimero patologico?

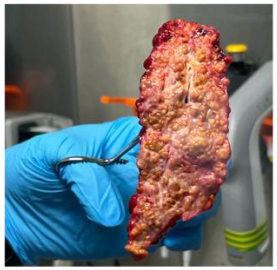


Lomas *et al*, *Nature* 1992; 357: 605-607

Yamasaki *et al*, *Nature* 2008; 455: 1255-1258

Yamasaki *et al*, *EMBO Rep.* 2011; 12: 1011-1017

Preparazione di polimeri intraepatici Z α_1-antitripsina



ZZ patient liver tissue

Thinly sliced

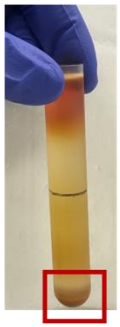
Treated with Collagenase Type 1A

Fibrous tissue removed

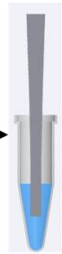
Supernatant sonicated and resuspended in 10% sucrose

Placed atop a 10-45% sucrose gradient

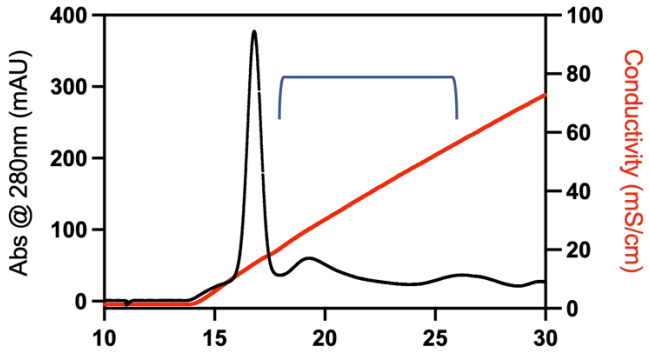
ultracentrifugation At 25000g 2 hours



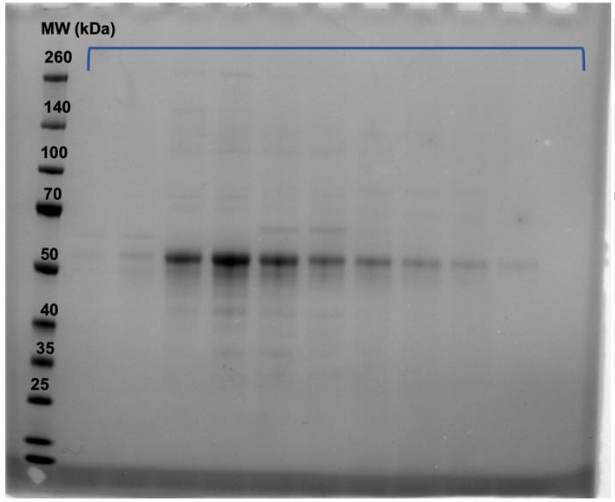
Inclusion bodies are washed several times



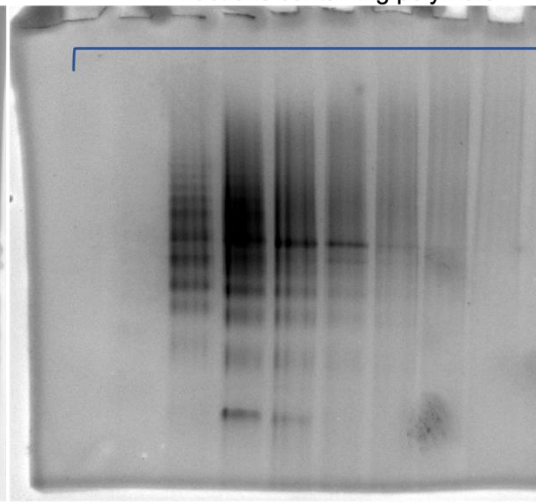
Sonicat releases s polyme



SDS-PAGE



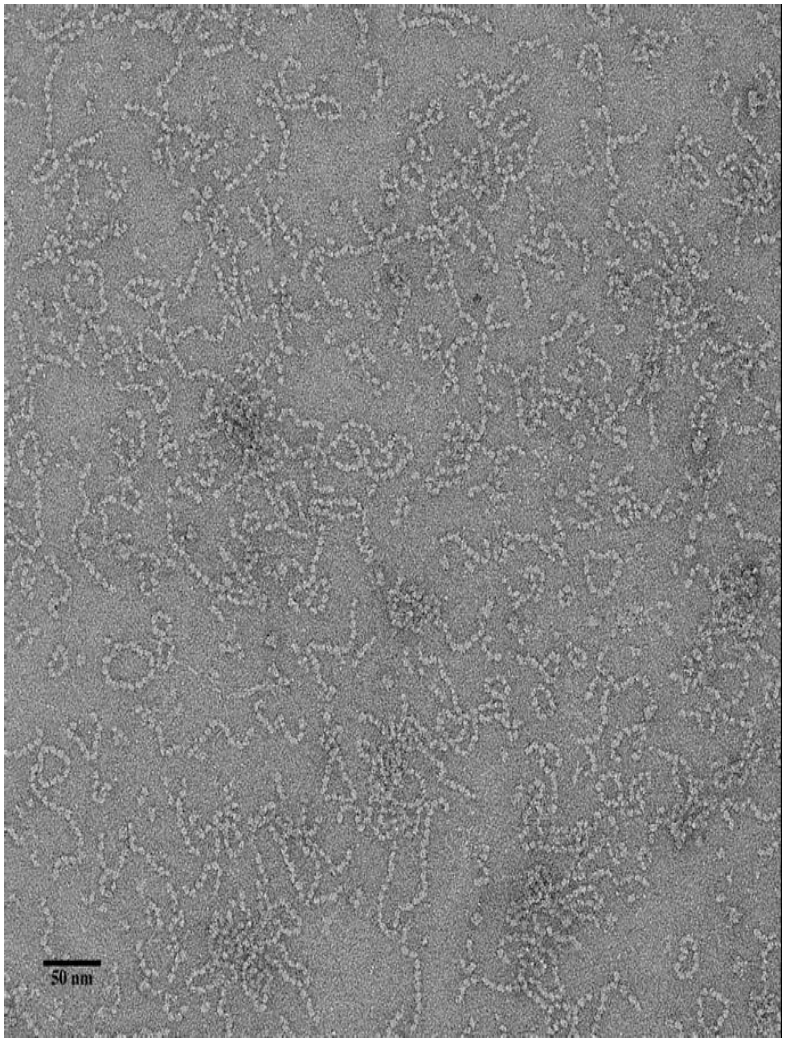
Native-PAGE used to identify fractions containing polymers



Ibrahim Aldobiyan

Imaging dei polimeri intraepatici Z α_1-antitripsina mediante microscopia elettronica

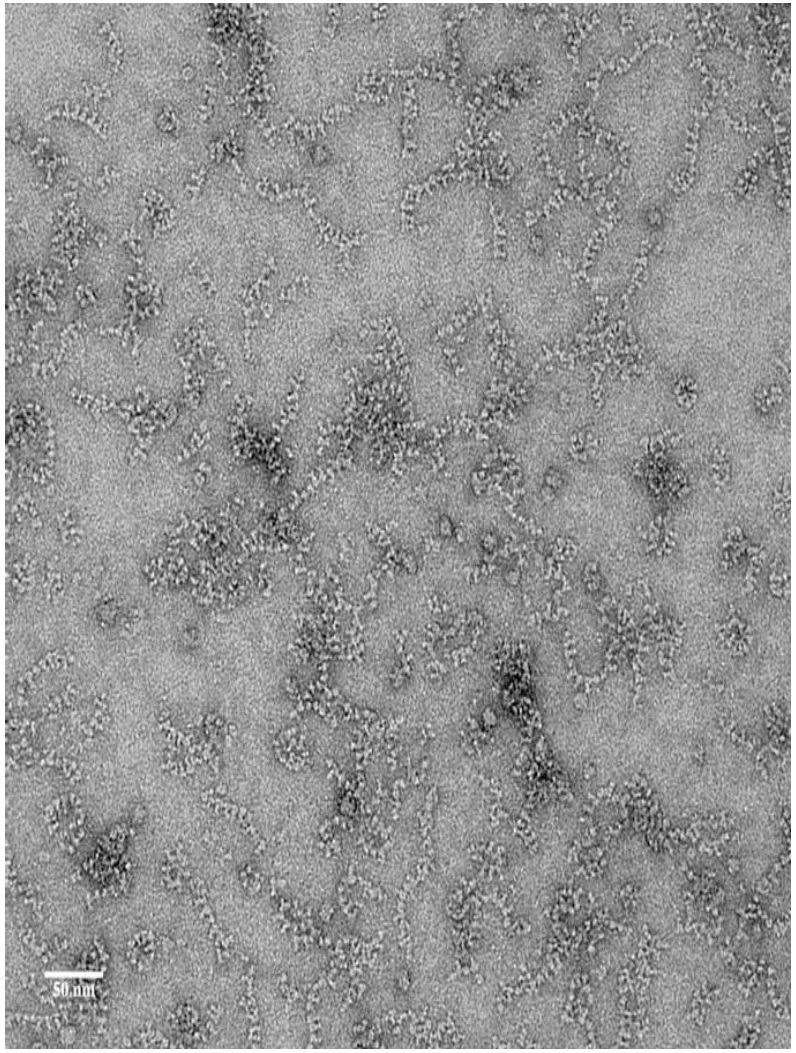
Negative stain

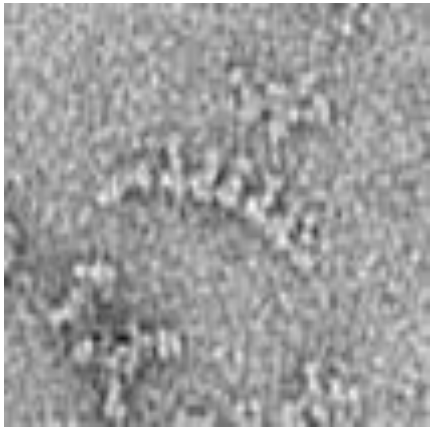


Emma Elliston

Imaging dei polimeri intraepatici Z α_1-antitripsina mediante microscopia elettronica

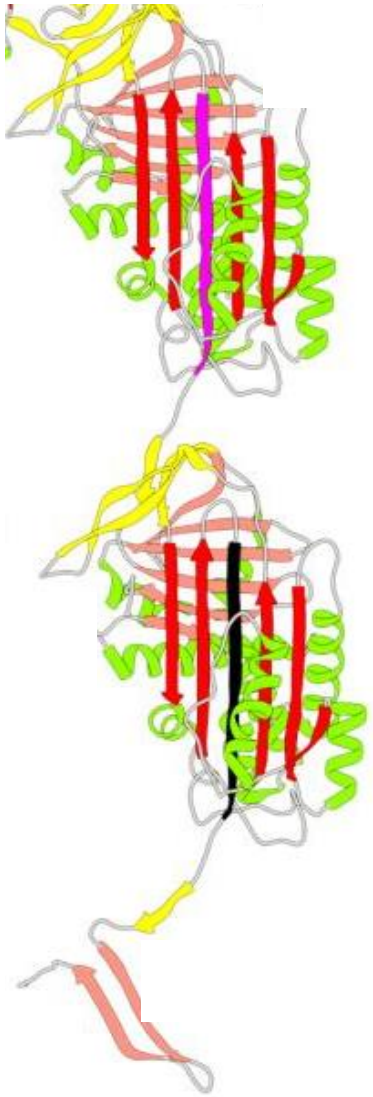
Negative stain of polymers labelled with 4B12 Fab





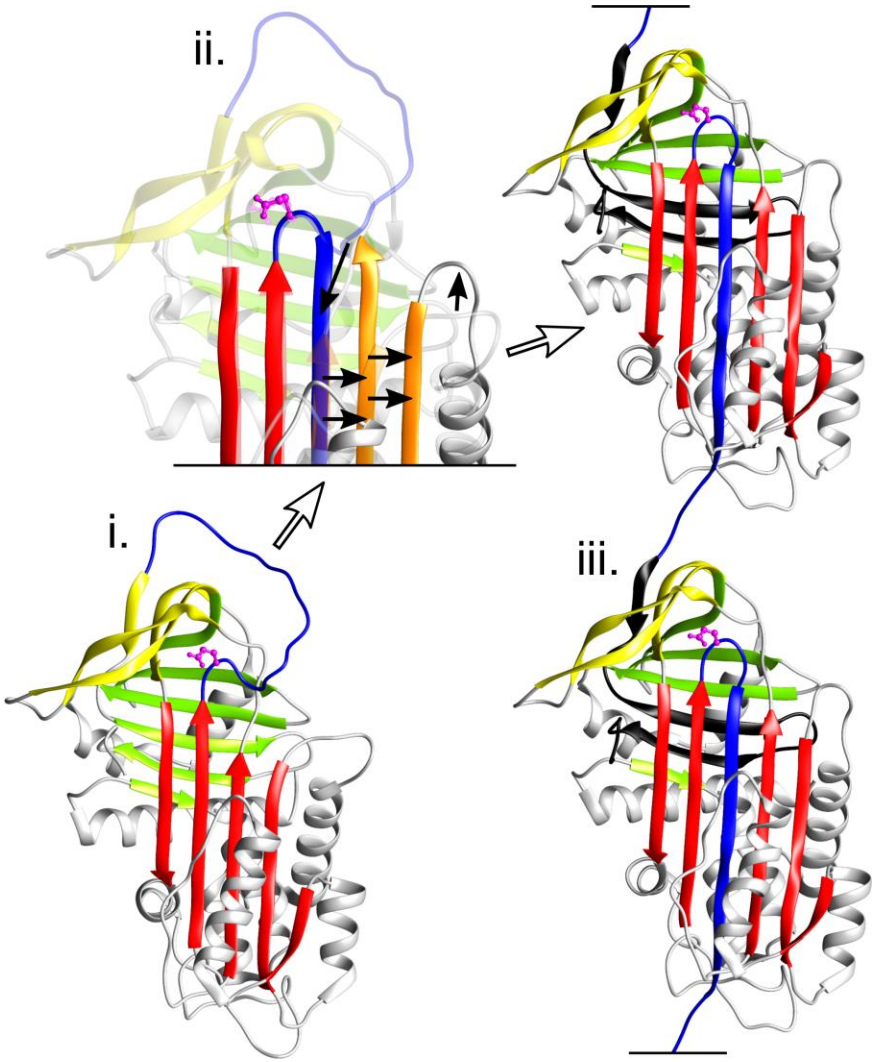
Emma Elliston

Il polimero patologico



Faull *et al*, *Science Advances* 2020; 21;6(43):eabc1370

Il polimero patologico

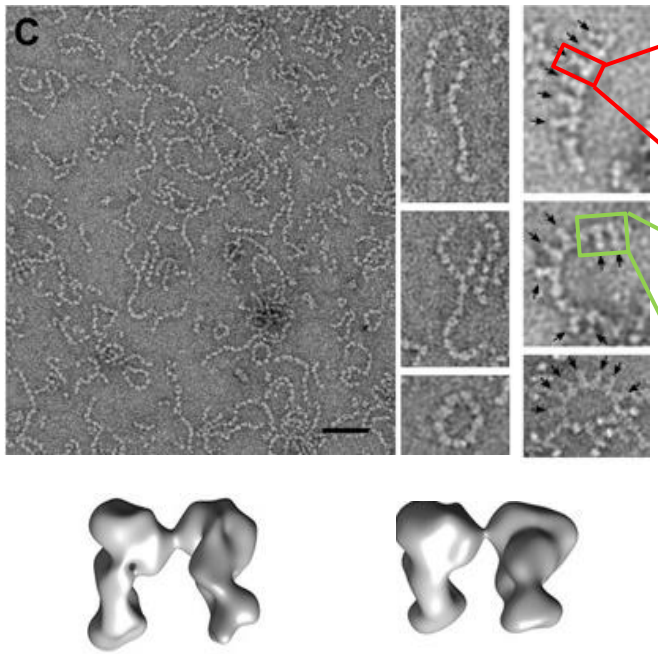


Faull *et al*, *Science Advances* 2020; 21;6(43):eabc1370

Verso una ricostruzione 3D ad alta risoluzione di polimeri AAT derivati dal paziente

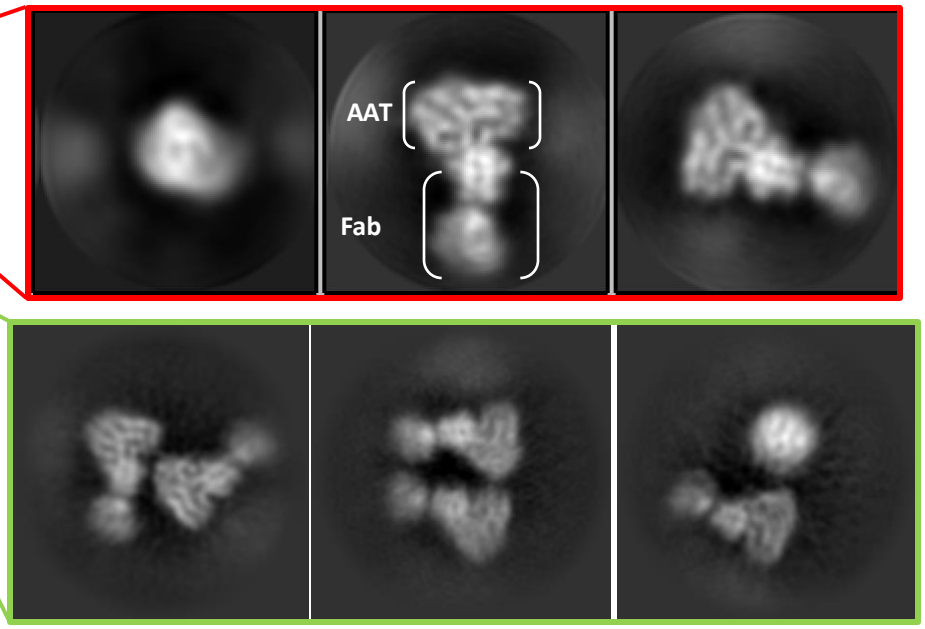
NSEM

ZZ AAT liver - Fab + Fab



Cryo-EM

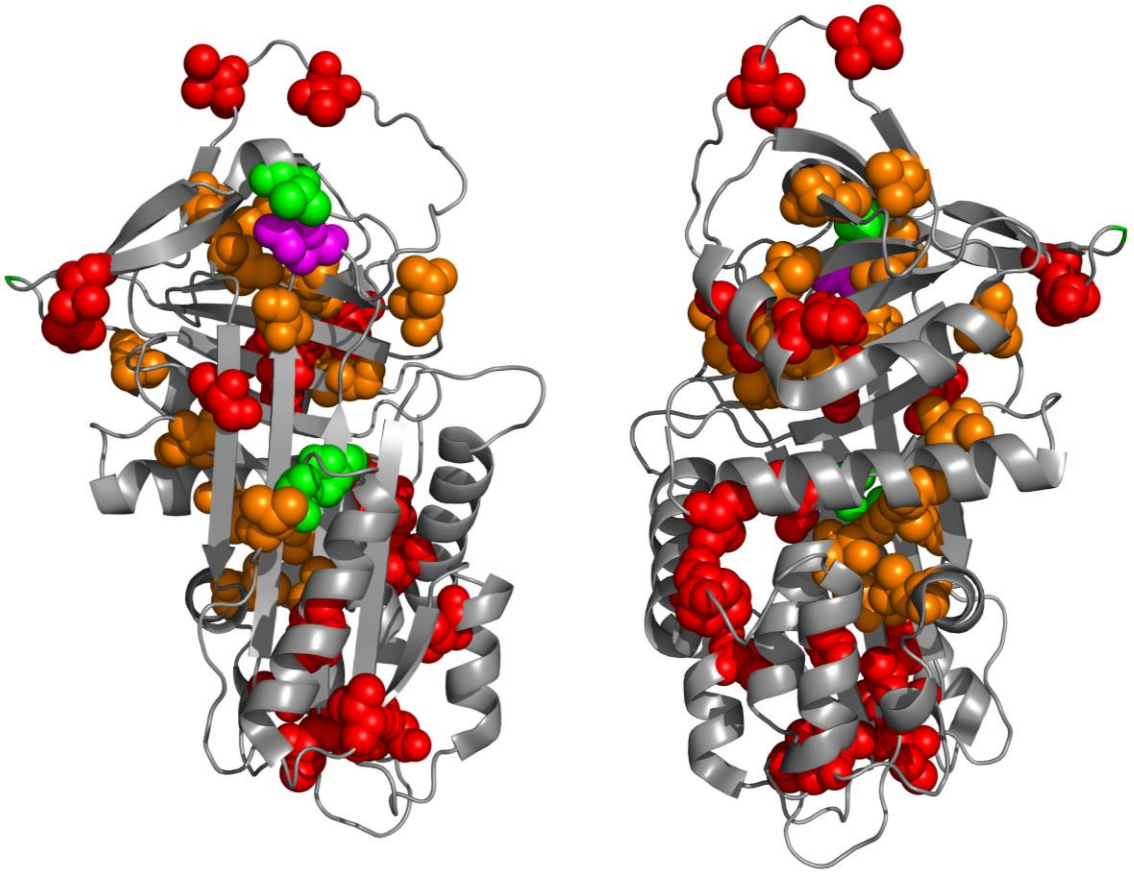
Preliminary data



Faull, SV. Science. 2020

Ibrahim Aldobiyan

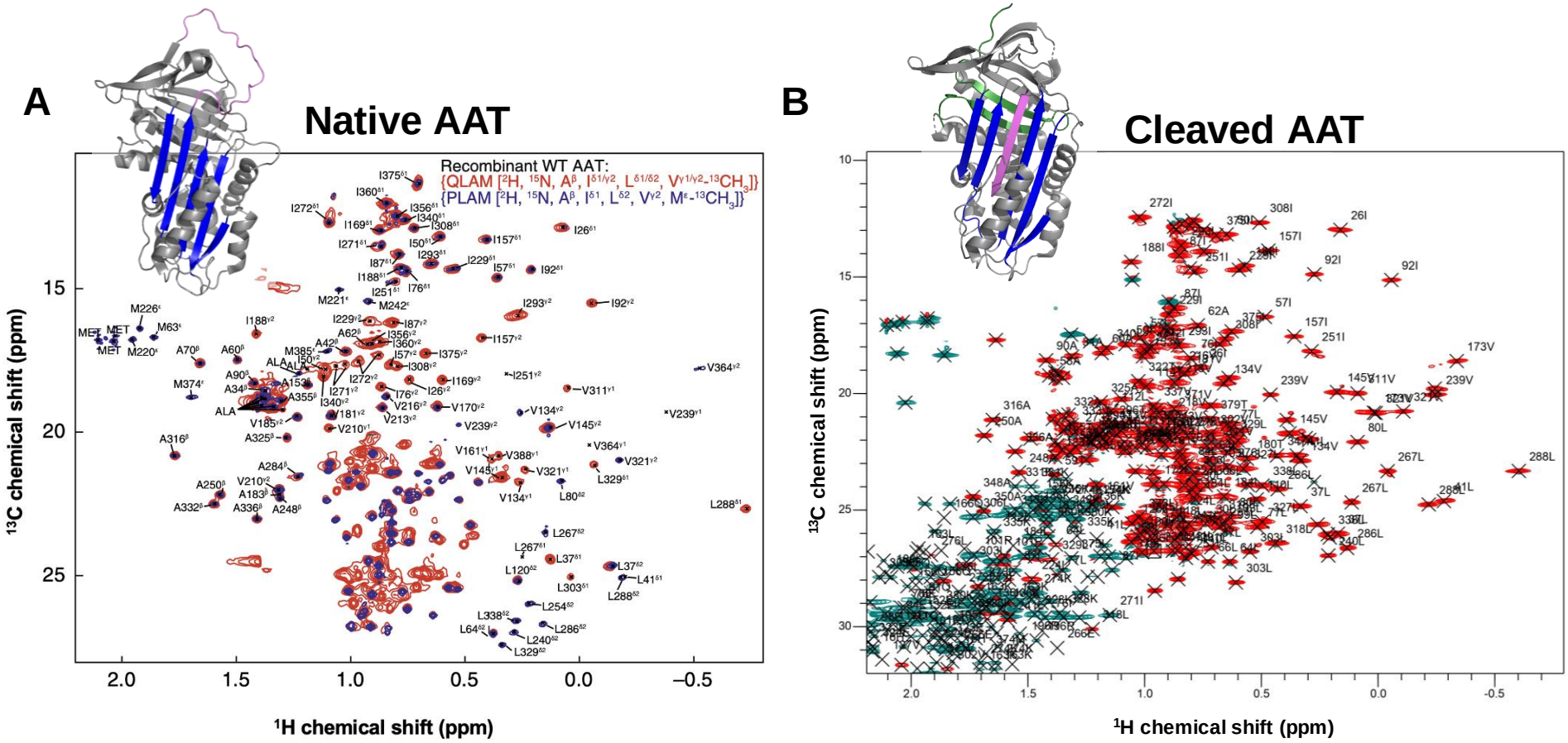
Abbondanza naturale NMR di M and Z α_1-antitripsina



Magenta = Glu342Lys mutation Red = No change to WT
Orange = Chemical shift change Green = Large shifts/loss

Jagger *et al*, *Nature Comms* 2020; 11(1):6371

L'assegnazione metilica di AAT può essere utilizzata come sonde

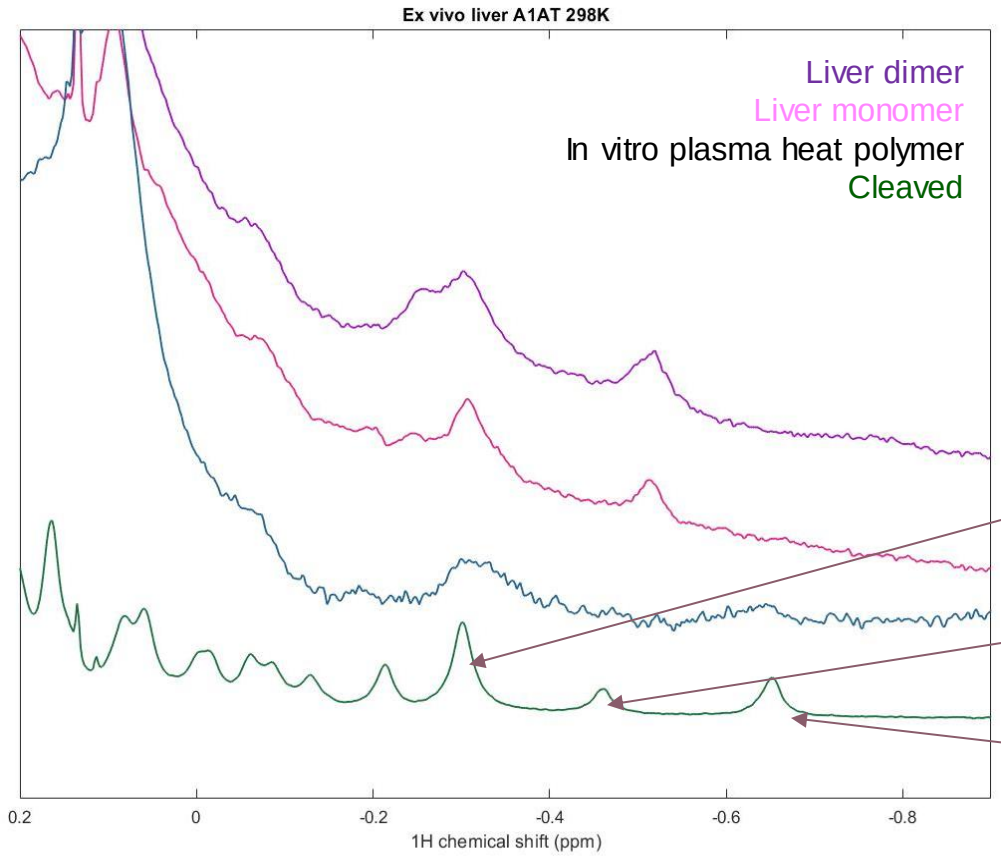


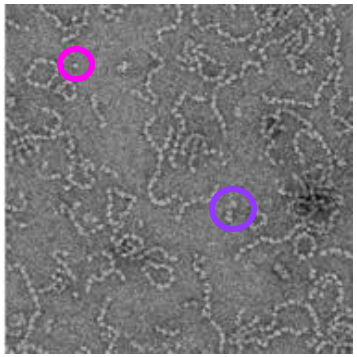
Jagger, A.M., Waudby, C.A., Irving, J.A. *et al. Nat Commun* **11**, (2020).

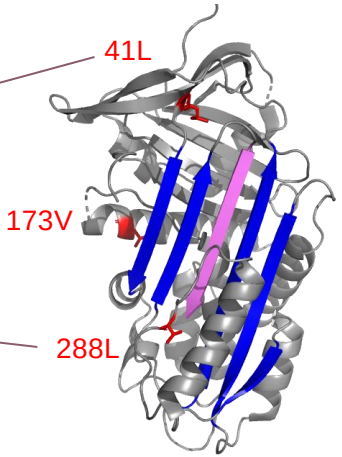
Sarah Lowen

NMR in stato di soluzione di AAT di fegato espiantato

^{1}H spectrum AAT methyl resonances







Repeat at higher temp and stronger magnet to resolve peaks and provide structural information

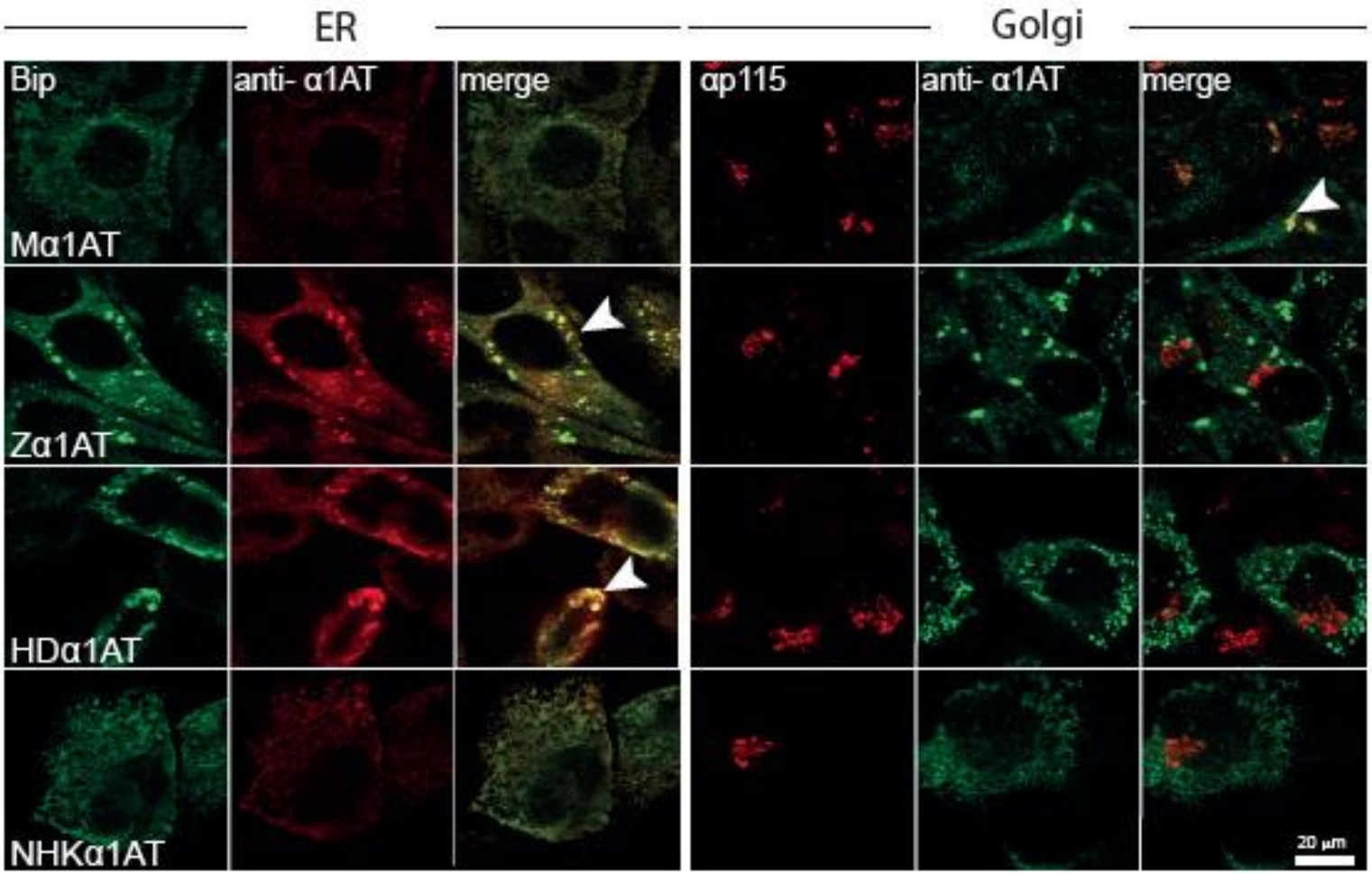
Sarah Lowen

Il deficit plasmatico di α_1-antitripsina è proporzionale alla velocità di polimerizzazione

	Mutation	Polymerisation	Deficiency
Z	Glu342Lys	+++	+++
Siiyama	Ser53Phe	+++	+++
Mmalton	Δ52Phe	+++	+++
King's	His334Asp	++++	+++
S	Glu264Val	+	+
I	Arg39Cys	+	+

Mahadeva *et al*, *J. Clin. Invest.* 1999; 103: 99-1006
Miranda *et al*, *Hepatology* 2010; 52: 1078-1088

I polimeri AAT sono trattenuti all'interno dell'ER



Miranda *et al*, *Hepatology* 2010; 52: 1078-1088

Ordóñez *et al*, *Hepatology* 2013; 57: 2049-60

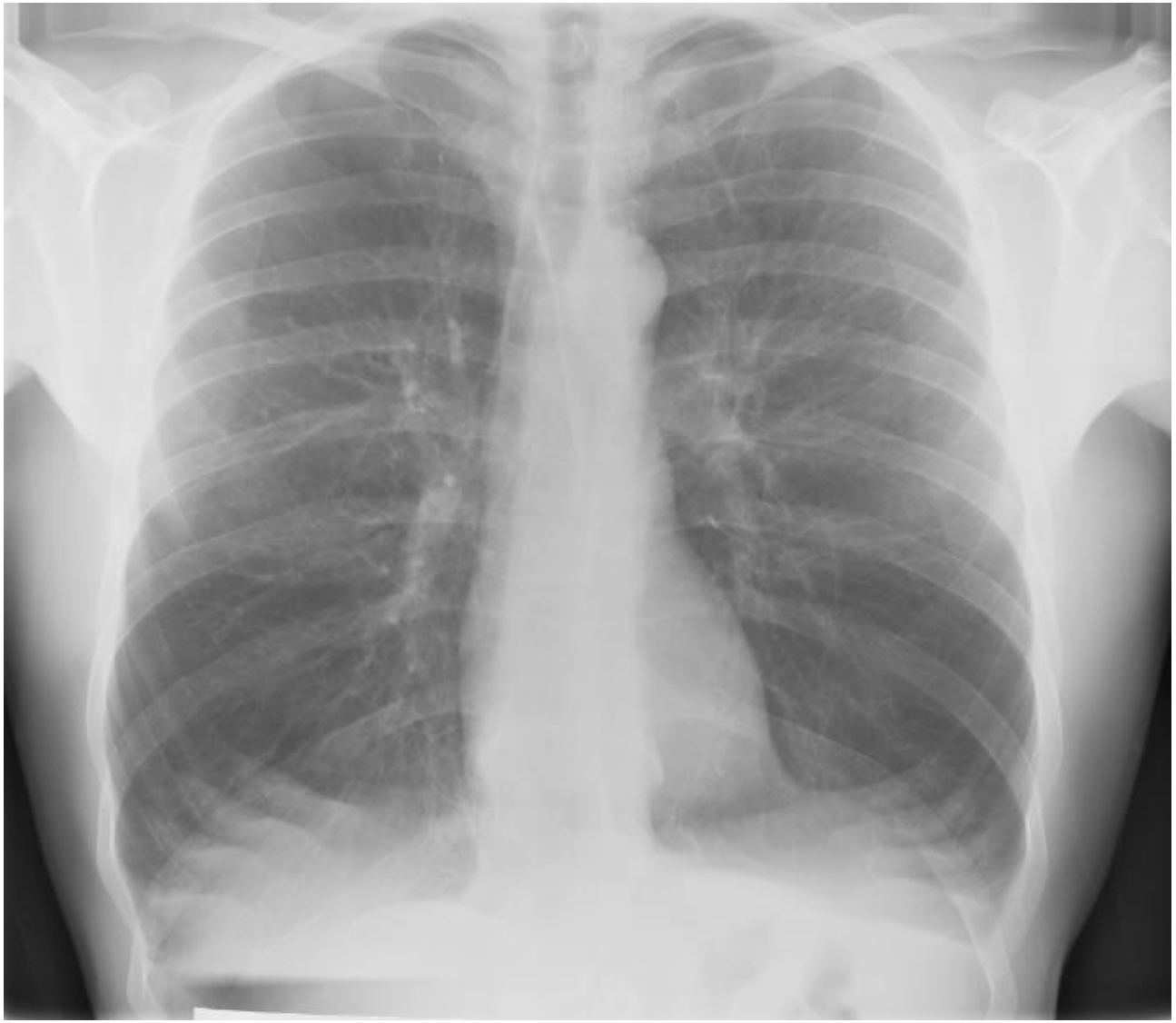
Conseguenze cellulari della polimerizzazione

Polymers do not induce the unfolded protein response (Bip, IRE-1, ATF-6, CHOP)

Polymers prime the cells to ER stress

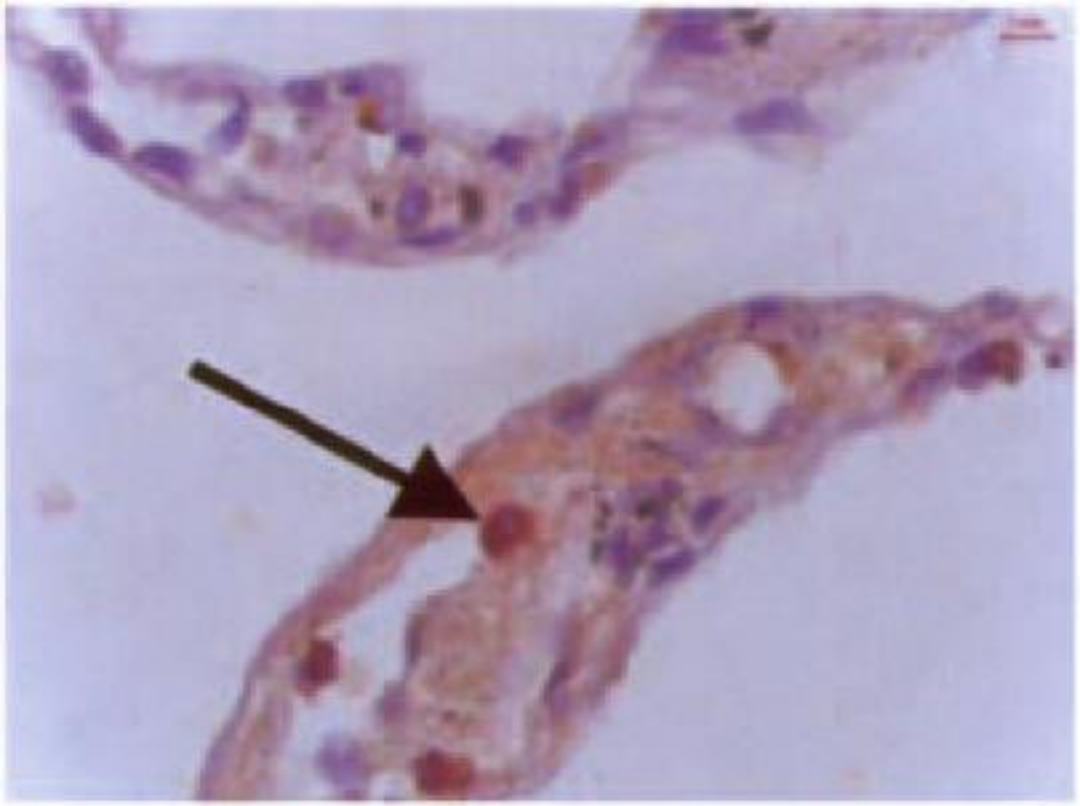
Ordóñez *et al*, *Hepatology* 2013; 57: 2049-60

Enfisema con deficit di α_1-antitripsina



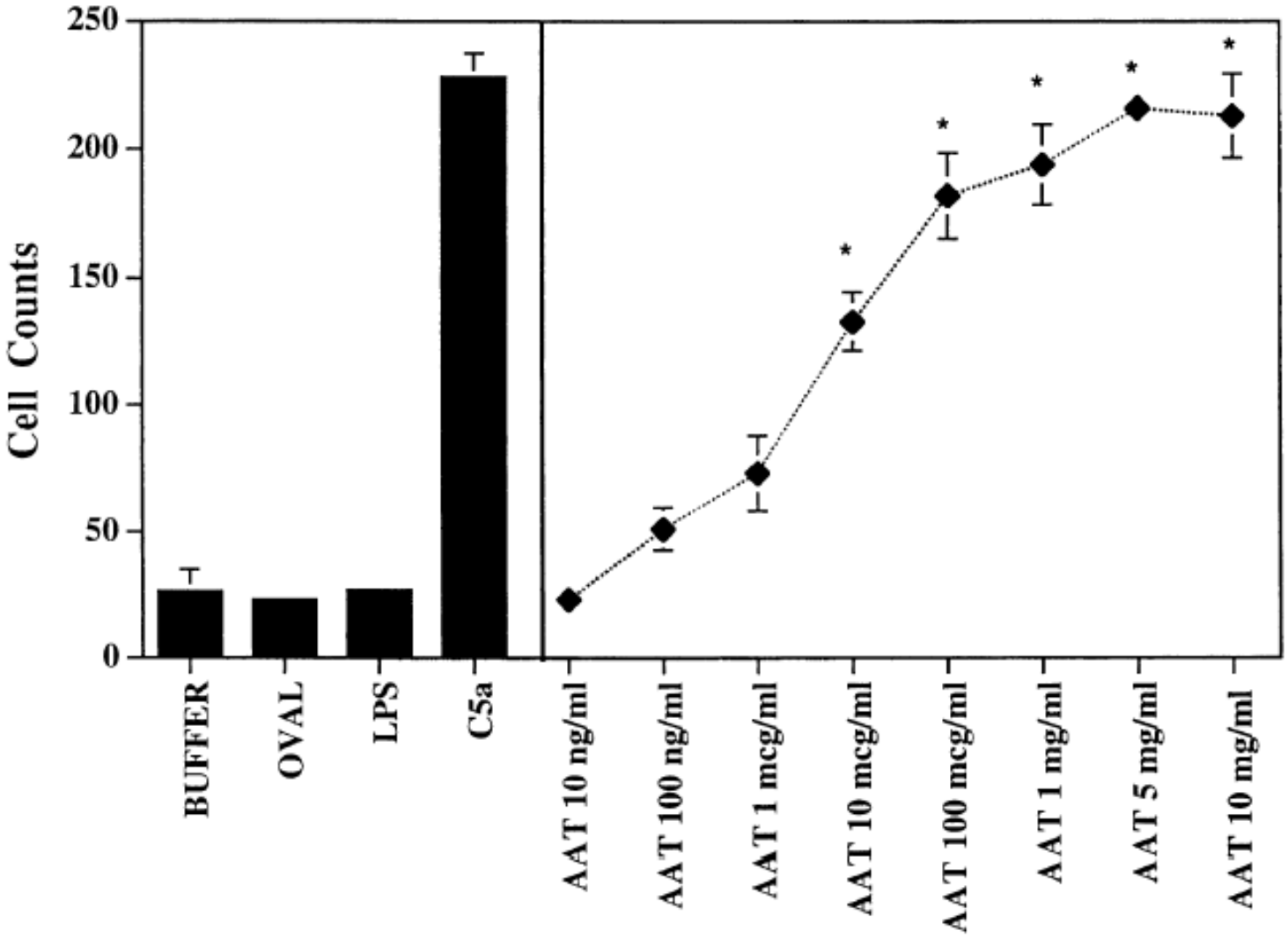
Eriksson, *Acta Med. Scand.* 1965; suppl 432: 1-85

I polimeri Z α_1-antitripsina co-localizzano con i neutrofili *in vivo*



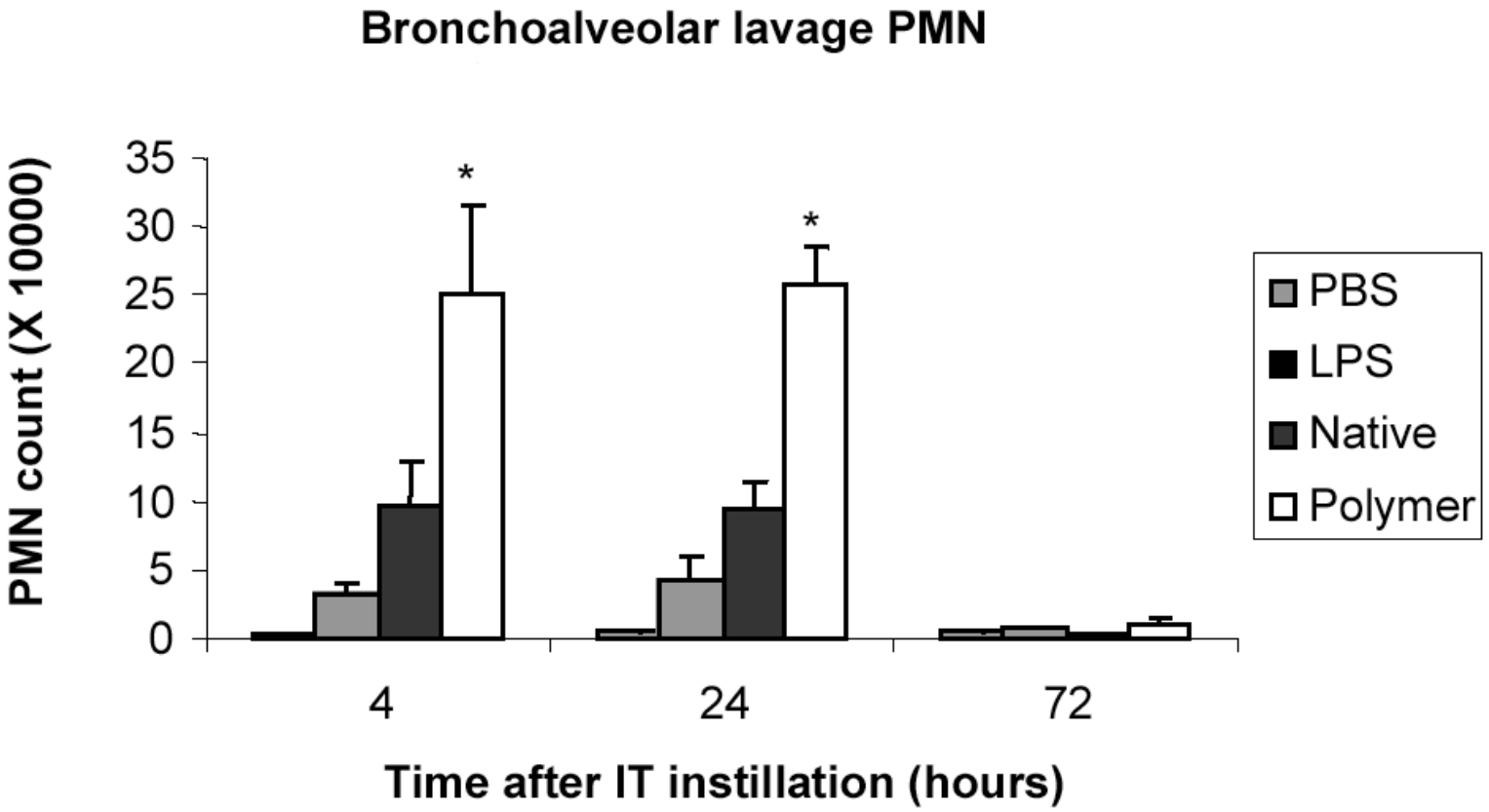
Mahadeva *et al*, *Am. J. Pathol.* 2005; 166: 377-386

I polimeri α_1-antitripsina sono chemiotattici *in vitro*



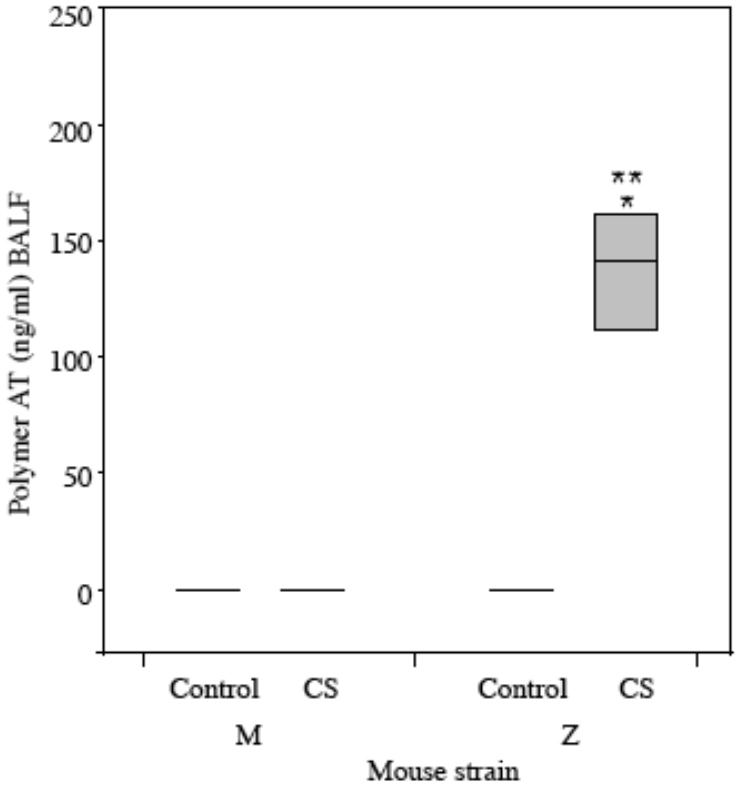
Parmar *et al*, *AJRCMB* 2002; 26: 723-730

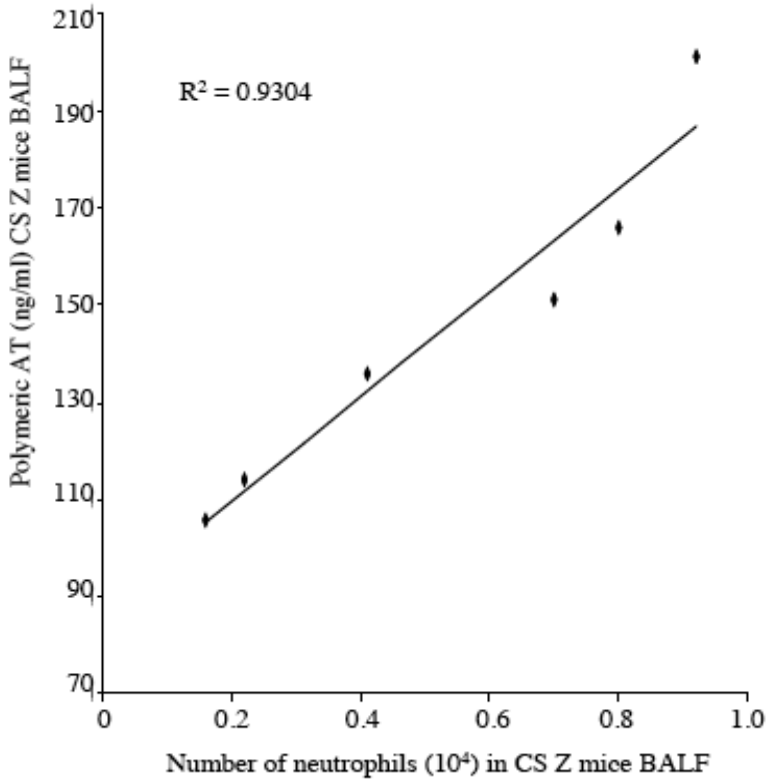
I polimeri α_1-antitripsina sono chemiotattici *in vitro*



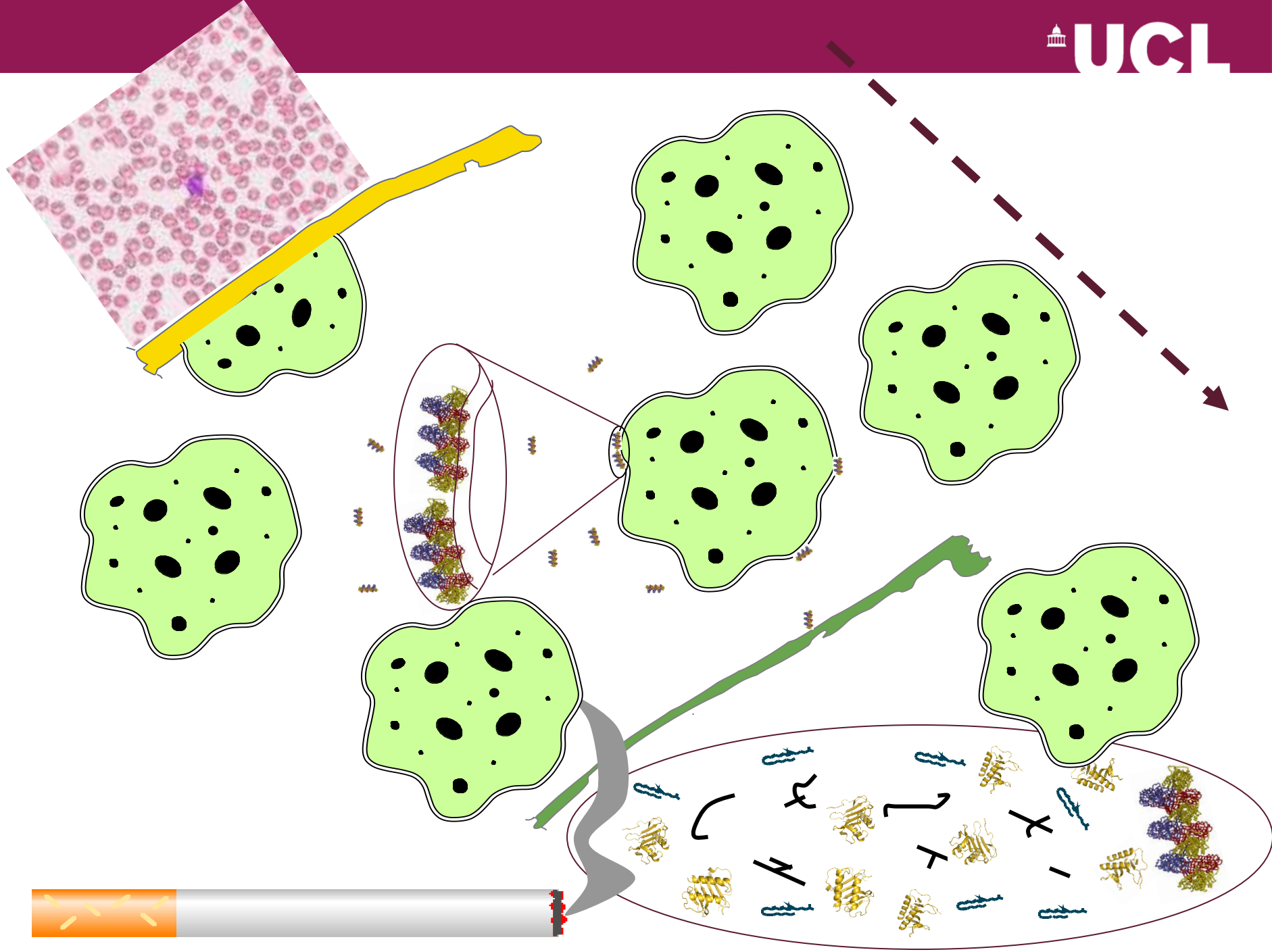
Mahadeva *et al*, *Am. J. Pathol.* 2005; 166: 377-386

Il fumo di sigaretta induce la polimerizzazione *in vivo*

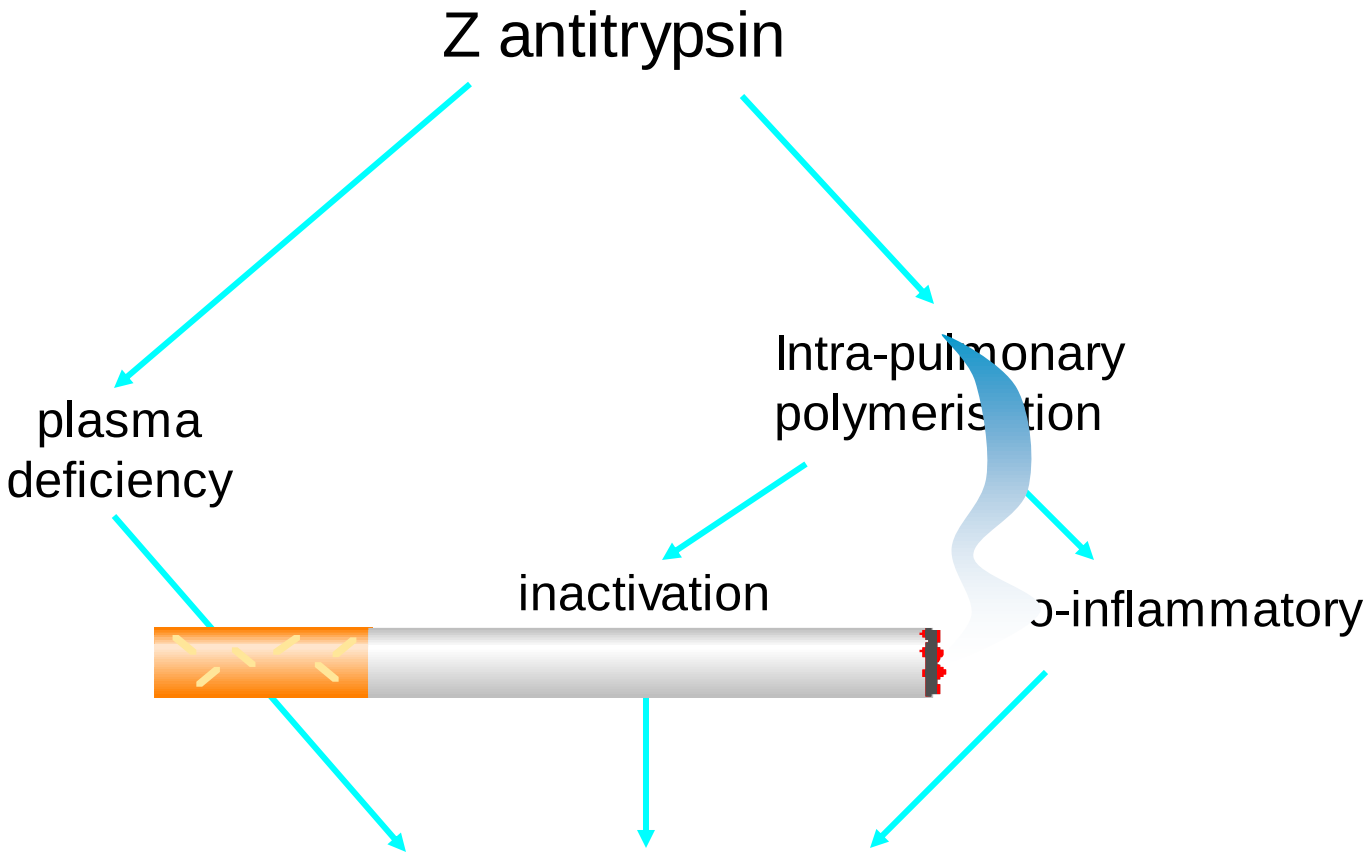




Alam *et al*, *AJRCMB* 2011; 45: 261-269



Meccanismi per la patogenesi dell'enfisema



Lomas and Mahadeva, *J.Clin. Invest.* 2002; 110: 1585-1590

Gooptu and Lomas, *J. Exp. Med.* 2008; 205: 1529-1534

Polimeri extraepatici di α_1-antitripsina

Polymers are present in the lung, skin and kidney of PiZZ homozygotes

Elliott *et al*, *Am. J. Resp. Cell Mol Biol.* 1998; 18:670-674

Gross *et al*, *Dermatology. J.* 2009; 218:370-375

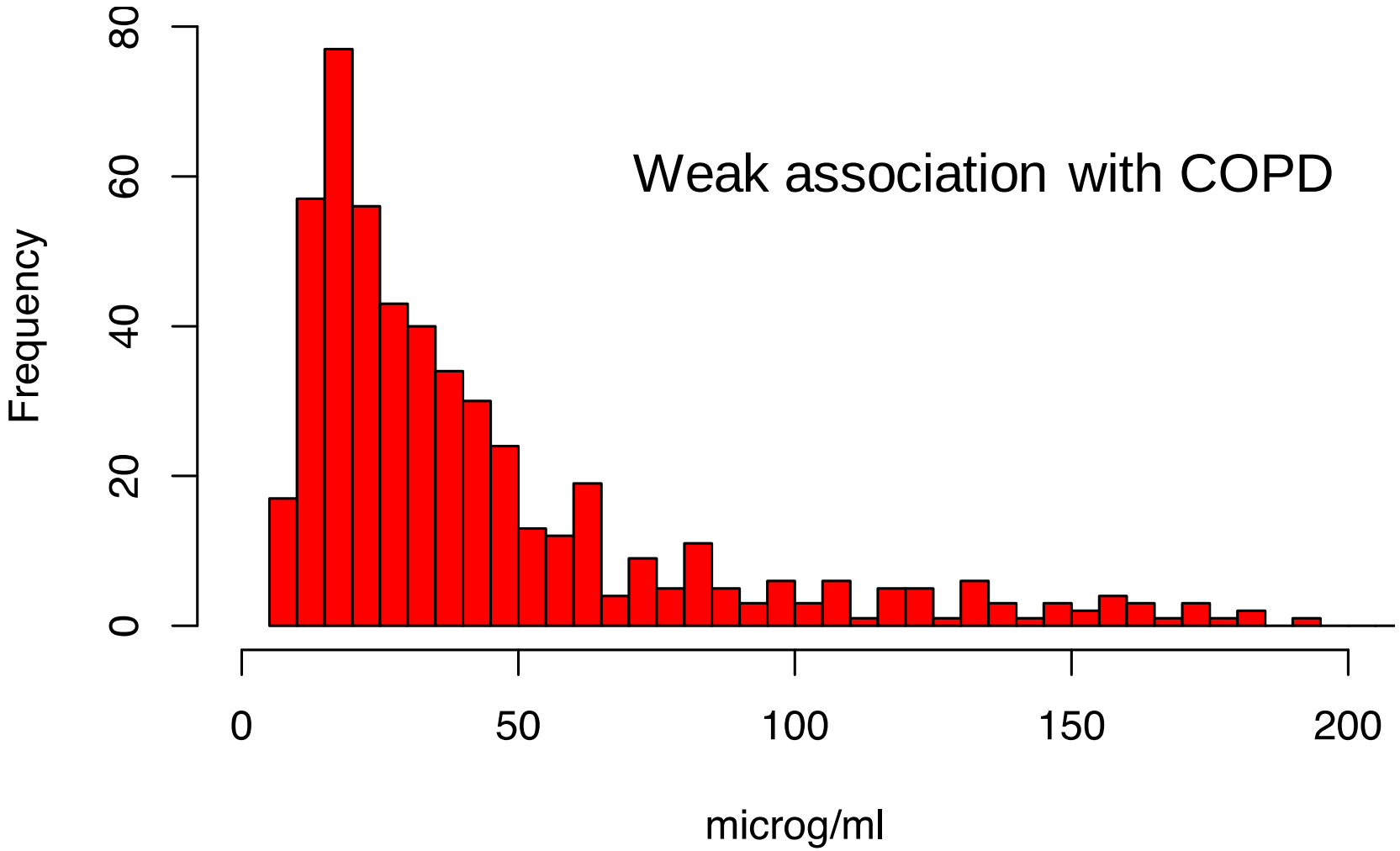
Morris *et al, Ann Rhem. Dis.* 2011; 70:1851-1856

Polimeri extraepatici di α_1-antitripsina

…and in the circulation of 517/517 PiZ homozygotes

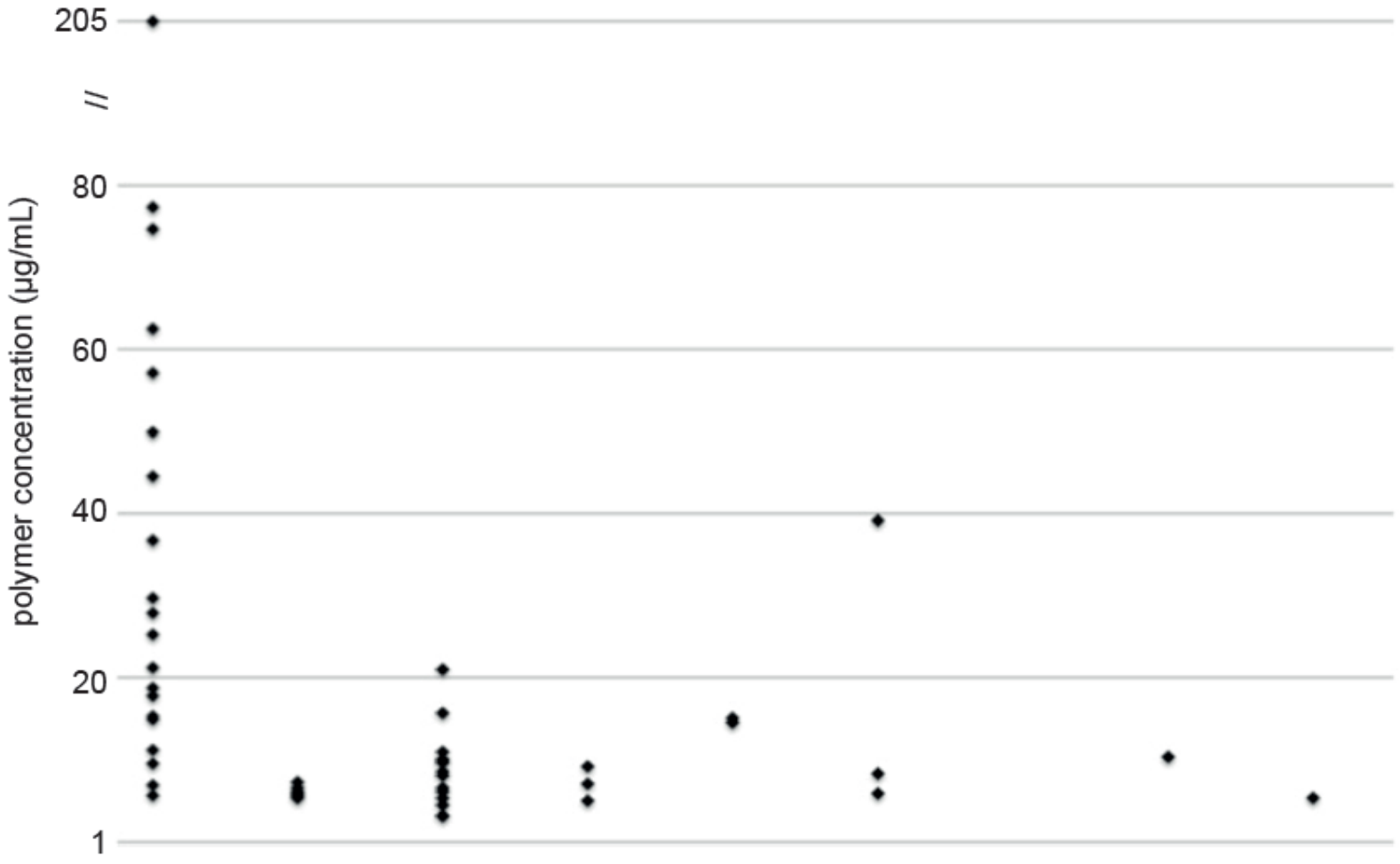
Tan *et al*, *Eur. Resp. J.* 2014; 45: 1501-1504

Polimeri circolanti di α_1-antitripsina



Tan *et al*, *Eur. Resp. J.* 2014; 45: 1501-1504

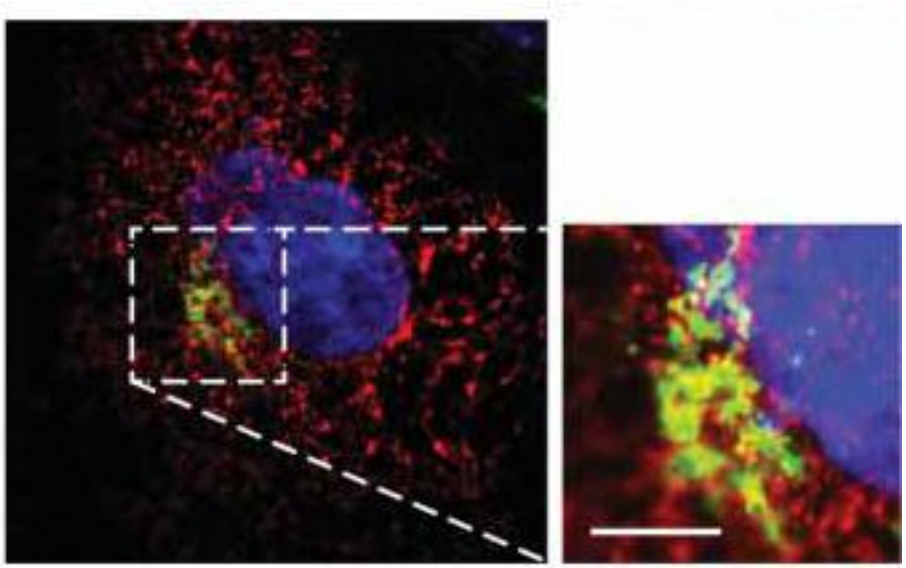
Genotipi α_1-antitripsina e polimeri circolanti



genotype	ZZ	MZ	SZ	FZ	ZMmalton	ZMheerlan	SS	MS	MM
positive samples	20/20	7/20	14/20	3/5	2/2	3/3	0/3	1/20	1/200

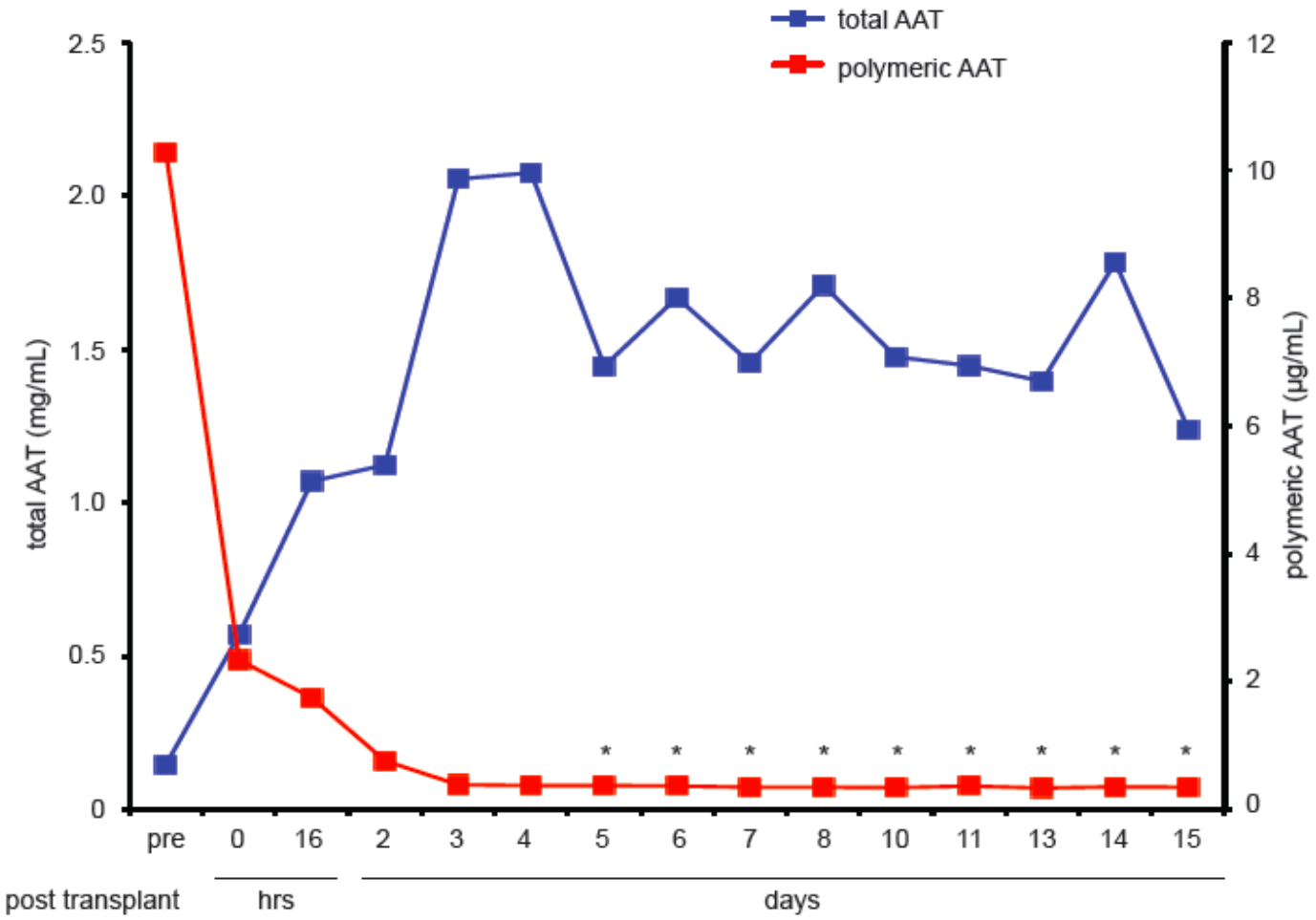
Vengono secreti i polimeri di Z α_1-antitripsina modelli cellulari attraverso il Golgi

Red = polymers
Green = TGN-46
Blue = nucleus



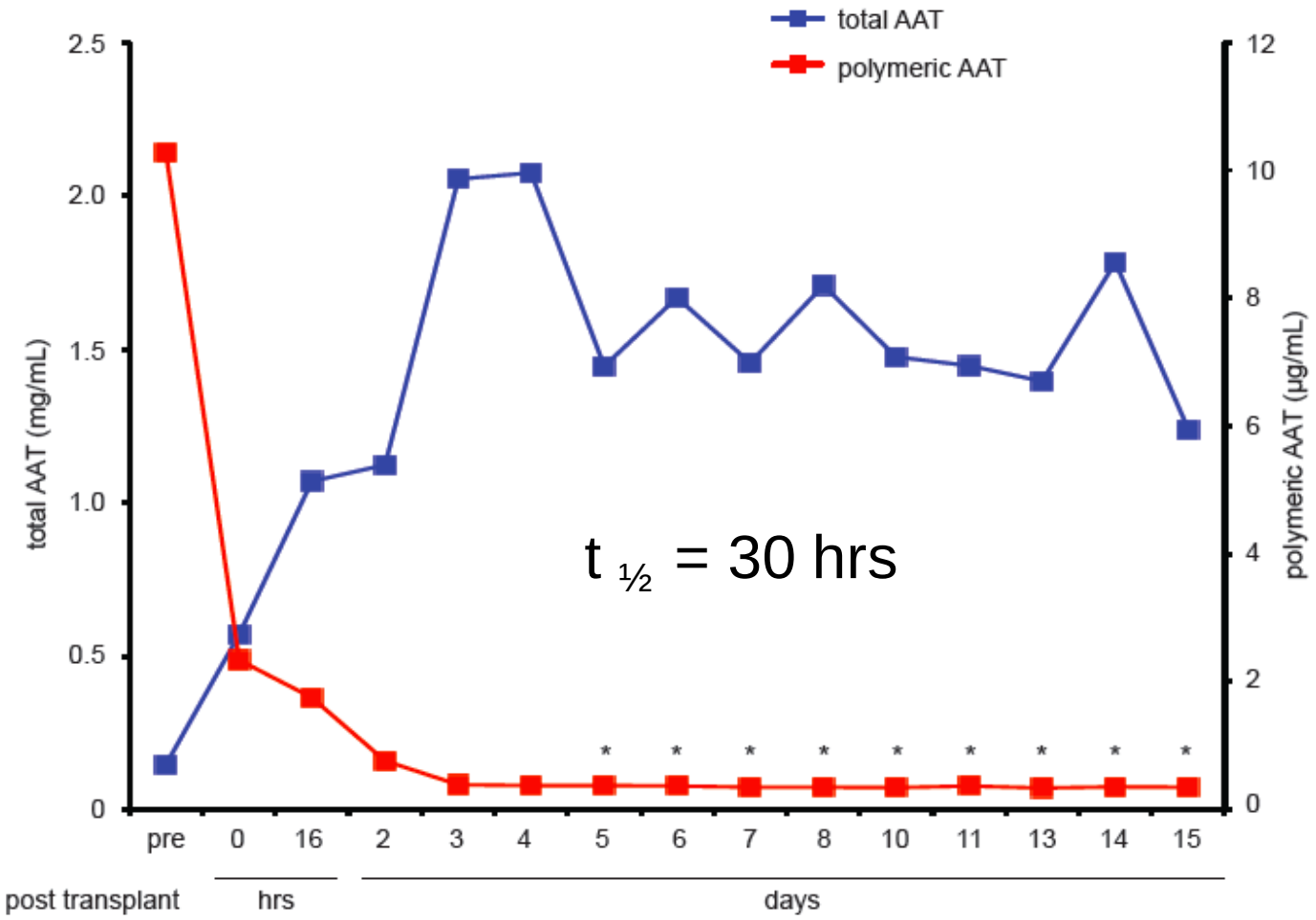
Fra *et al*, *Eur. Resp. J.* 2016; 47: 1005-9

I polimeri circolanti scompaiono dopo il fegato trapianto



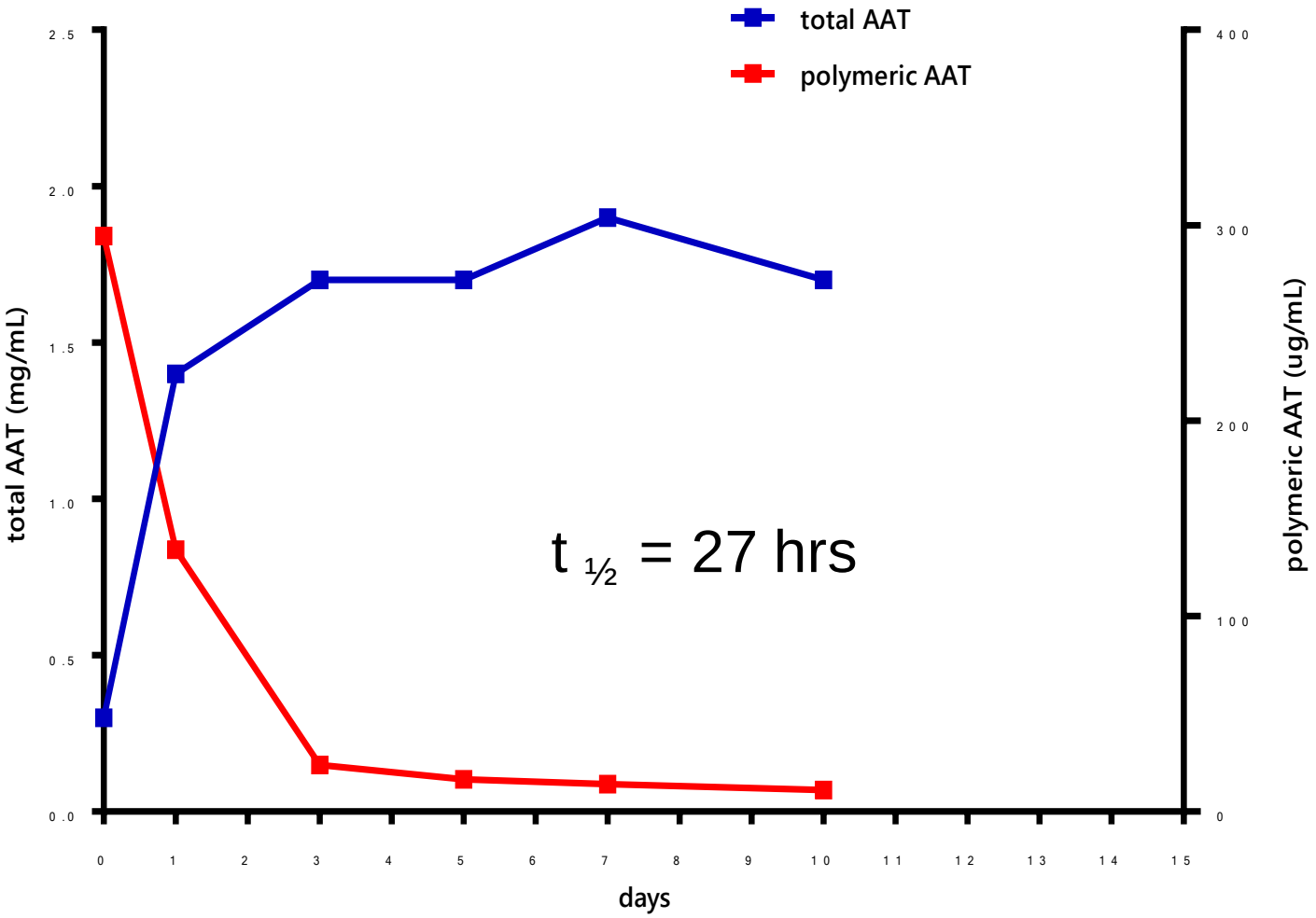
Tan *et al*, *Eur. Resp. J.* 2014; 43: 1501-4

I polimeri circolanti scompaiono dopo il fegato trapianto



Tan *et al*, *Eur. Resp. J.* 2014; 43: 1501-4

I polimeri circolanti scompaiono dopo il fegato trapianto



Nina Heyer-Chauhan

Polimeri come biomarker per il deficit di α_1-antitripsina

Childhood Liver Disease Research and Education Network (ChiLDREN).

Prospective study of 400 children with α_1-antitrypsin deficiency

Mean circulating polymer in cohort was 8.35 (SD+/-7.34) µg/ml

Higher polymers in children with portal hypertension (p=0.004),

Each 1 µg/ml increase in polymer level increased the likelihood of portal hypertension by 6.7%.

No correlation of total α_1-antitrypsin with portal hypertension

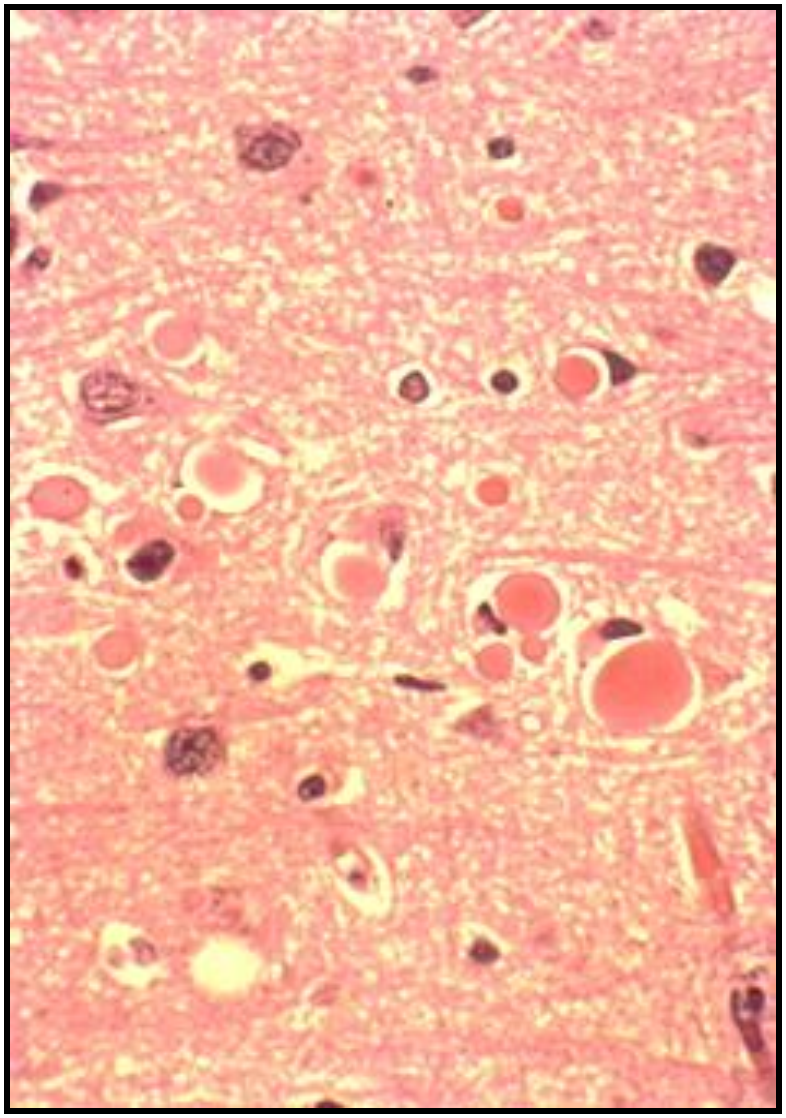
Teckman *et al*, AASLD meeting, Boston Nov 2014

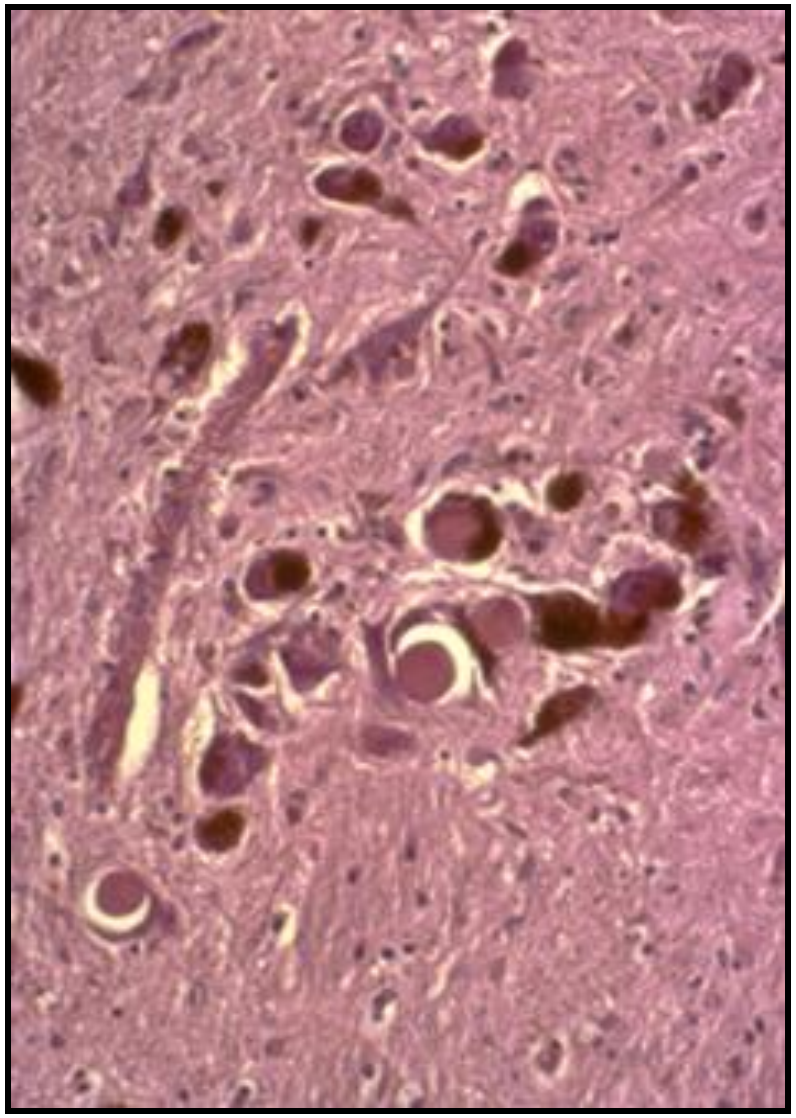
Inibitori della serina proteinasi o serpine

α_1-antitrypsin	inhibits neutrophil elastase
Antithrombin	inhibits enzymes of coagulation cascade
C1-inhibitor	inhibits enzymes of fibrinolysis
α_1-antichymotrypsin	inhibits enzymes of inflammation
Neuroserpin	inhibits tPA in neuronal development/ memory

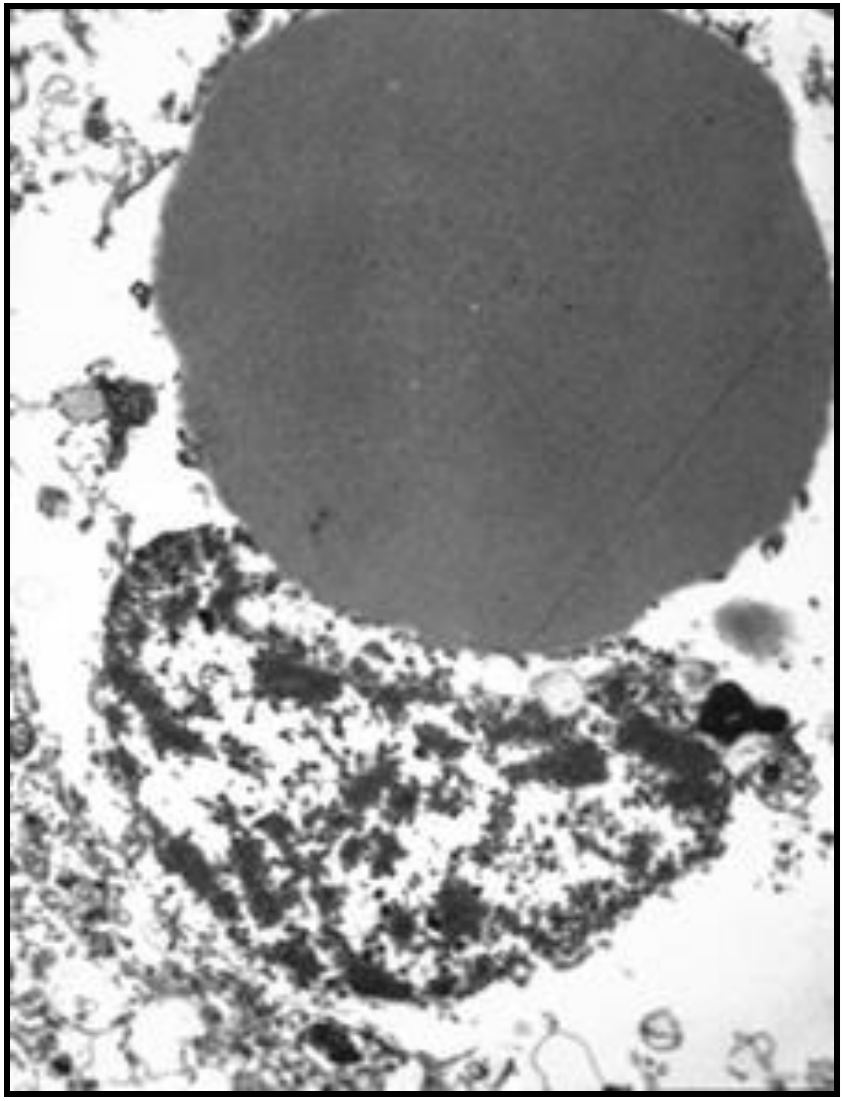
Gooptu and Lomas, *An. Rev. Biochem.* 2009; 78: 147-176

I corpi di inclusione della neuroserpina si colorano in modo positivo con PAS

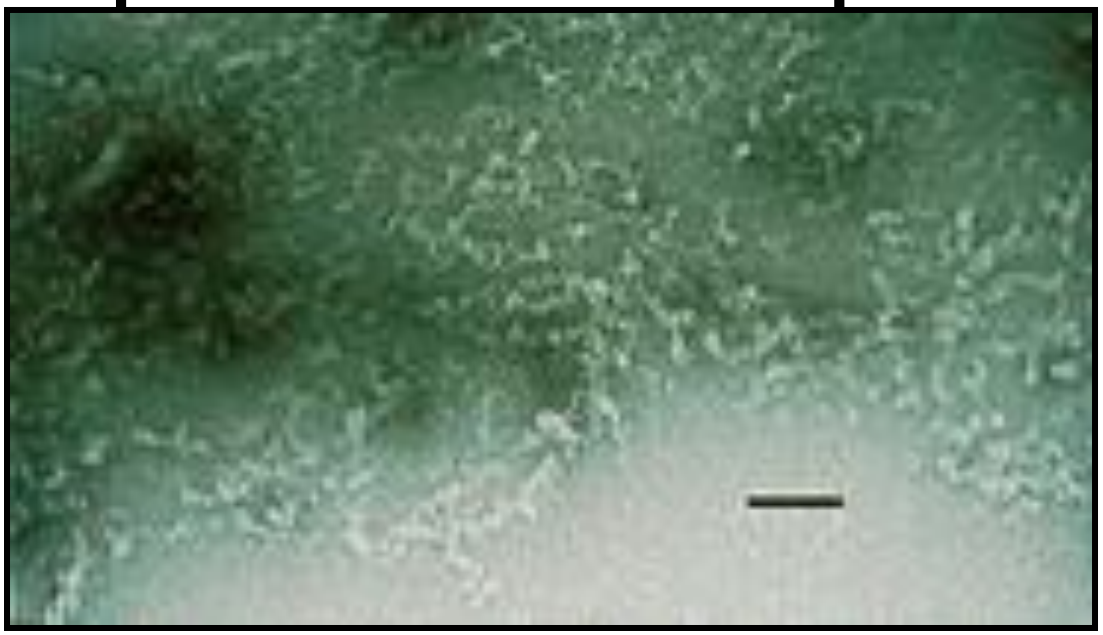


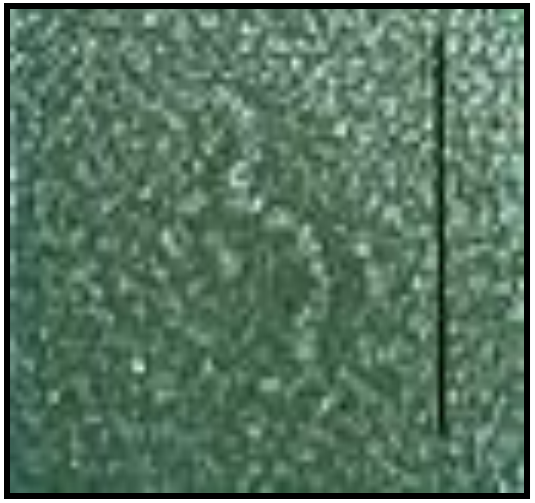


I corpi di inclusione della neuroserpina si trovano all'interno dell'ER



Le inclusioni sono formate da polimeri di neuroserpina





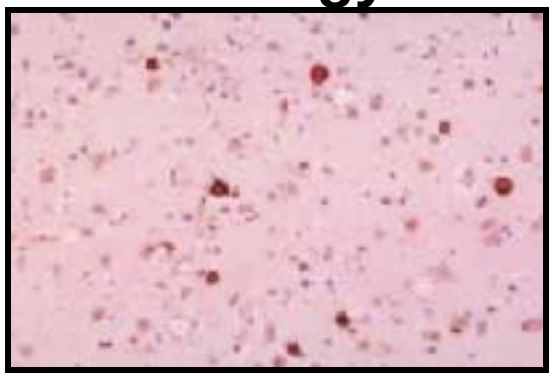
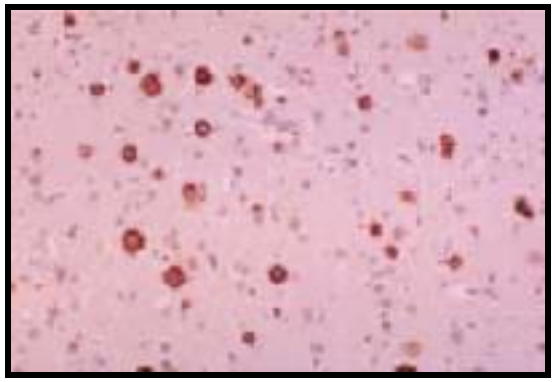
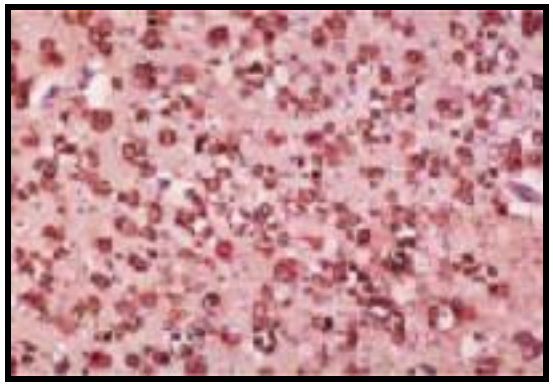
Familial encephalopathy with neuroserpin inclusion bodies (FENIB)

Davis *et al*, *Nature* 1999; 40: 376-379

Onset (yrs)	Clinical findings	Inclusions	Mutation
45-63	Dementia, seizures	+	S49P
20-40	Myoclonus, dementia	+ +	S52R/L47P
15	Progressive myoclonus epilepsy	+ + +	H338R
13	Progressive myoclonus epilepsy, chorea	+ + + +	G392E
8	Epilepsy with slow wave sleep	+ + + +	G392R

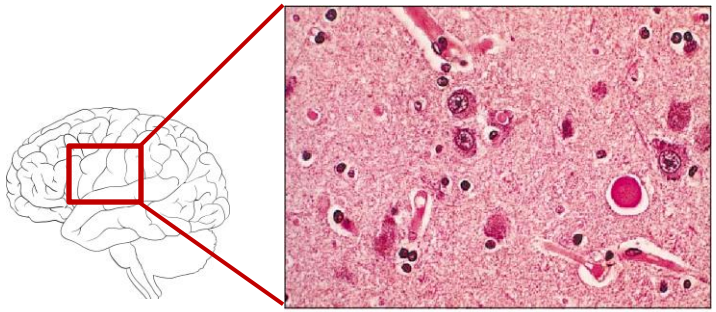
Davis *et al*, *Lancet* 2003; 359: 2242-2247

Coutelier *et al*, *Neurology* 2008; 71: 64-66 Hagen et al, *Brain Pathol.* 2011; 21:575-582

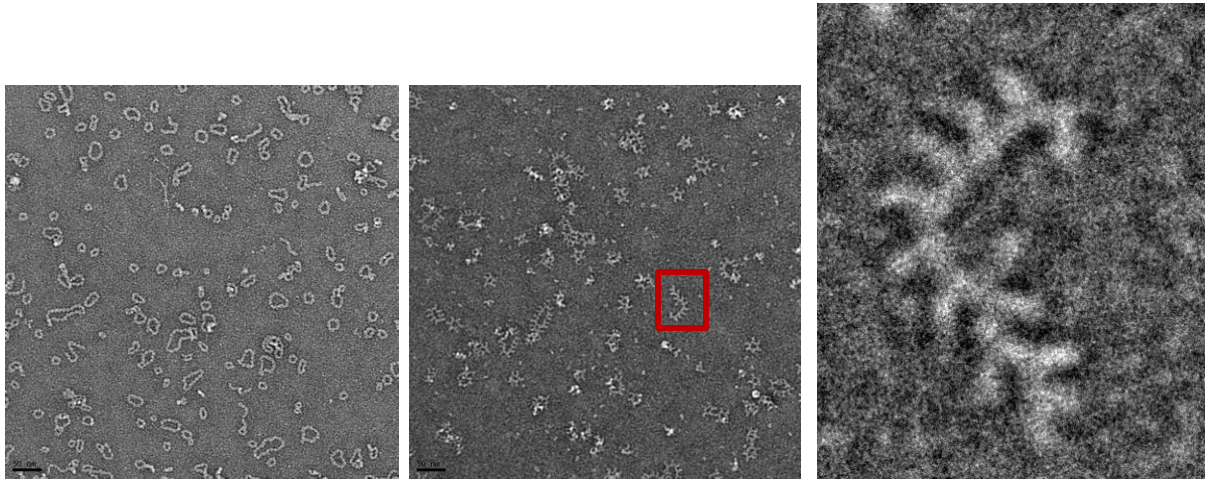
Onset (yrs)	Clinical features	Histology	Mutation
48 yr	Dementia, seizures terminally		S49P
24 yr	Myoclonus, status epilepticus, dementia		S52R
13 yr	Myoclonus, status epilepticus, dementia		G392E

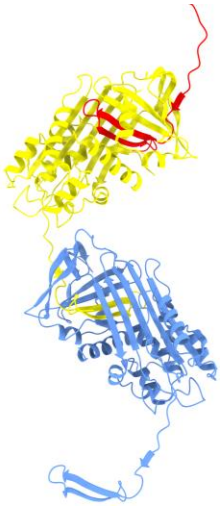
Davis *et al*, *Lancet* 2003; 359: 2242-2247

Sondaggio dell'universalità del legame nei polimeri



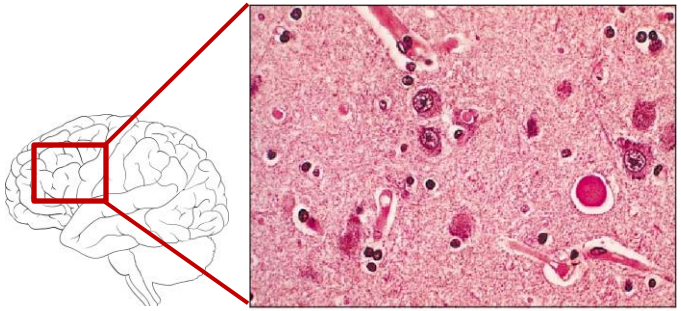
Ibrahim Aldobiyan



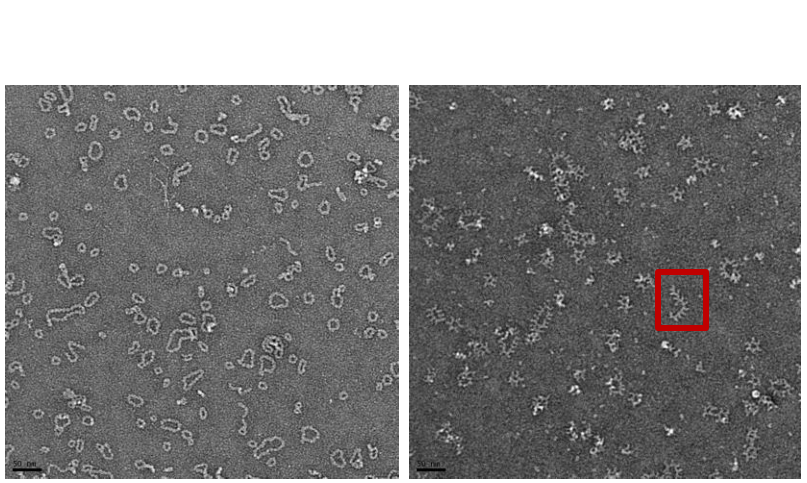


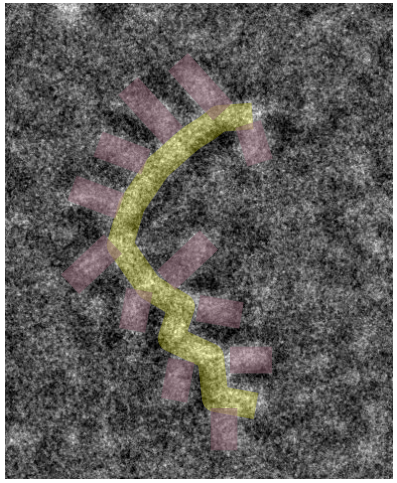
hNeuroserpin (syracuse) polymers ± 1A10 fab
(67000x magnification)

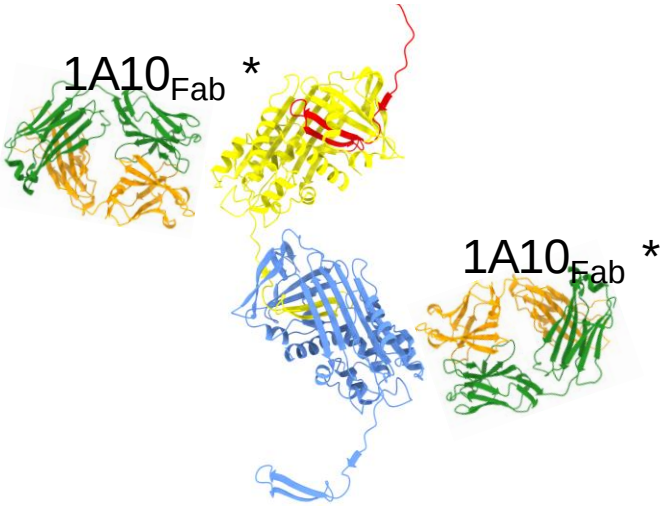
Sondaggio dell'universalità del legame nei polimeri



Ibrahim Aldobiyan







hNeuroserpin (syracuse) polymers ± 1A10 fab
(67000x magnification)

The Serpinopathies/Le Serpinopatie

Gain of function:	cirrhosis with Z antitrypsin
	dementia with neuroserpin (FENIB)
Loss of function:	thrombosis with antithrombin deficiency
	angio-oedema with C1 inhibitor deficiency
	emphysema with antichymotrypsin deficiency
	heparin co-factor II deficiency ?disease

Lomas and Mahadeva, *J. Clin. Invest*. 2002; 110: 1585-1590
Gooptu and Lomas, *J. Exp. Med.* 2008; 205: 1529-1534

Terapia per il deficit di α_1-antitripsina

Lung disease: intravenous augmentation therapy with plasma-purified AAT.

Liver disease: No therapy is currently approved for liver disease associated with AAT deficiency other than transplantation for advanced disease.

Strnad *et al*, *New Eng. J. Med.* 2020; 382: 1443-55

siRNA per silenziare la sintesi di Z α_1-antitripsina all'interno degli epatociti

N=16 in
2 doses

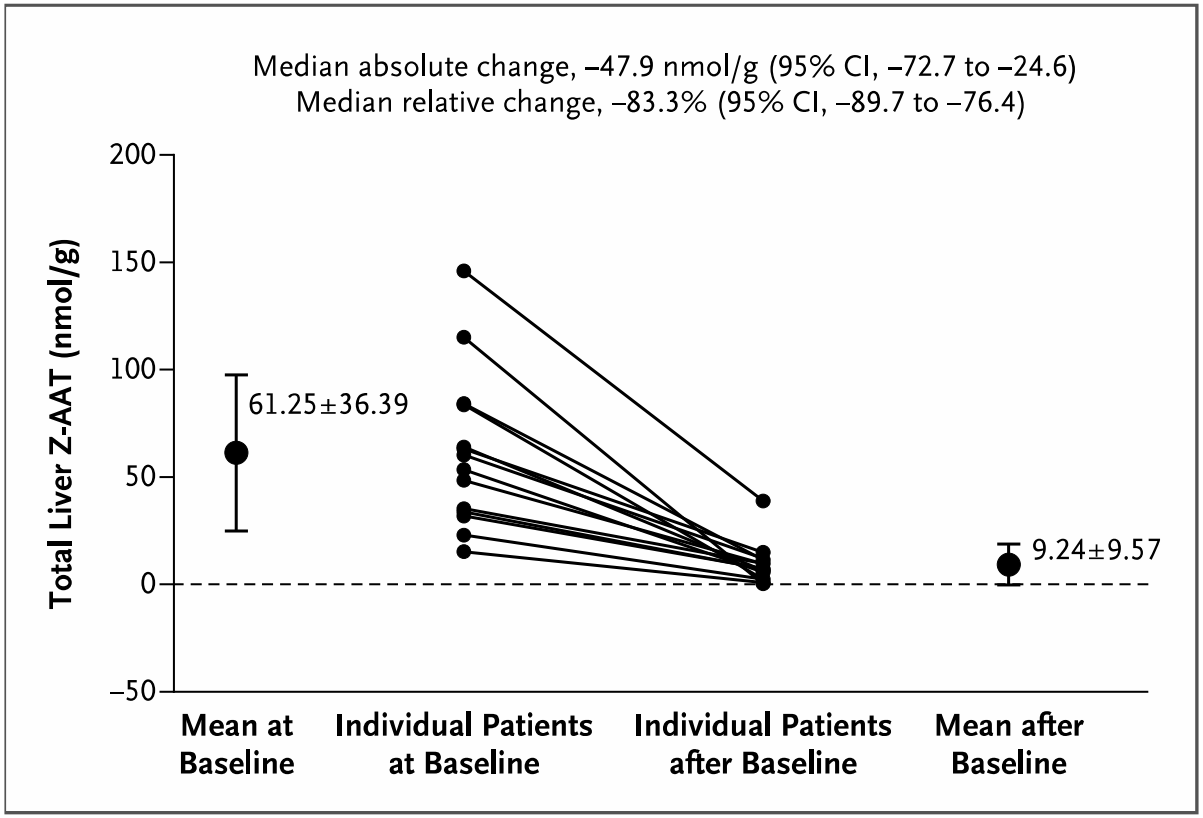
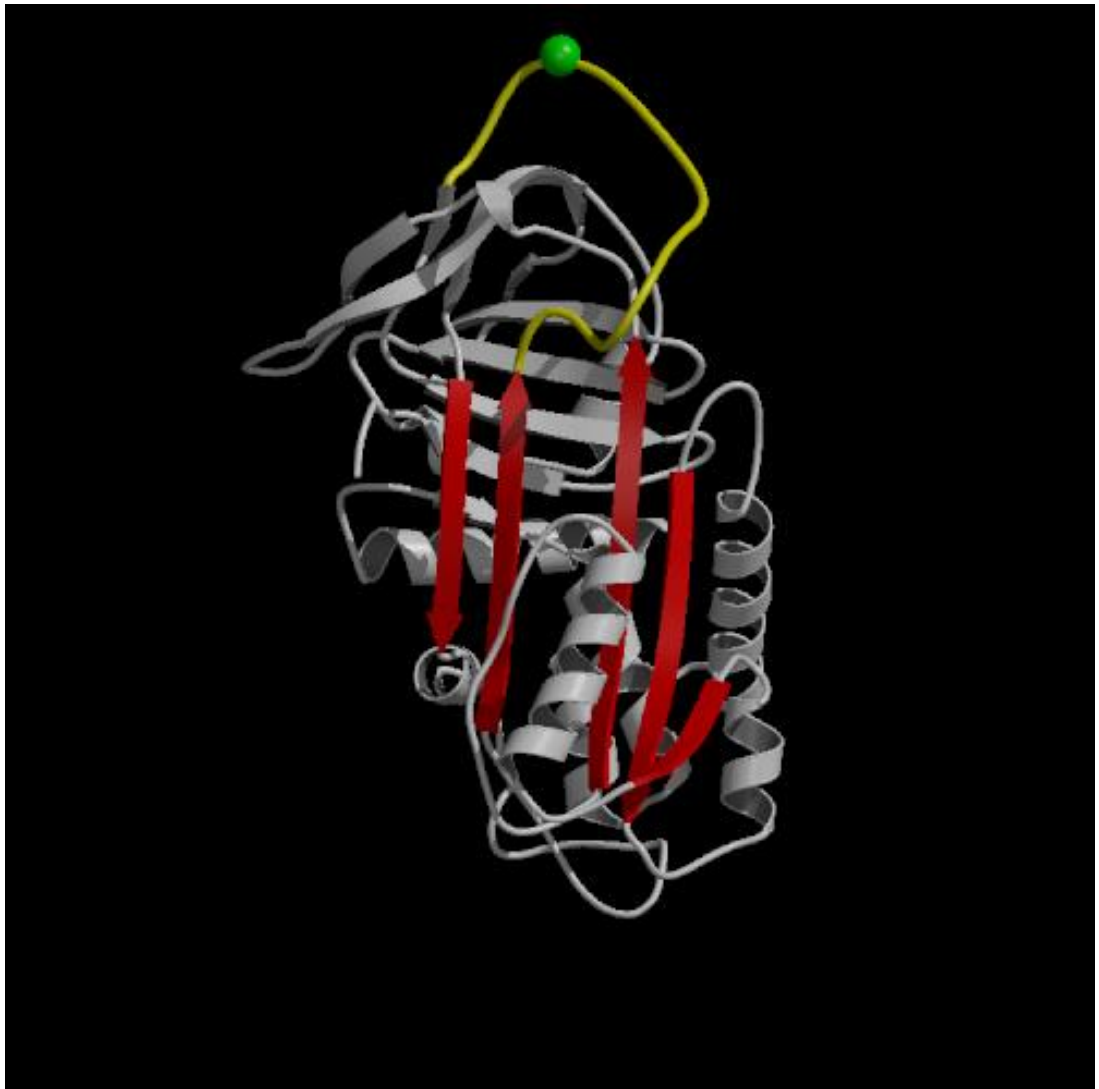


Figure 1. Effect of Fazirsiran Treatment on Liver Z-AAT Concentration at Week 24 or 48.

Strnad *et al*, *New Eng. J. Med.* 2022; 387: 514-524

Prevenzione della polimerizzazione di piccole molecole



Sfide nello sviluppo di bloccanti polimerici a piccole molecole con GSK

(i) the drug target is a highly mobile folding intermediate in the endoplasmic reticulum;

(ii) prevention of a large protein-protein interaction;

(iii) oral dosing greatly restricts suitable chemical space;

(iv) as a non-classical drug target, small molecule binders may well not be well-represented in compound screening libraries;

(v) the relatively concentration of circulating monomeric Z α_1-antitrypsin (~5μM) in PiZ homozygotes is a high affinity sink

Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

Approccio allo sviluppo di bloccanti polimerici a piccole molecole

Screen to block Z α_1-antitrypsin polymerisation *in vitro*

⬇

Assess leads in CHO cell model that expresses Z α_1-antitrypsin

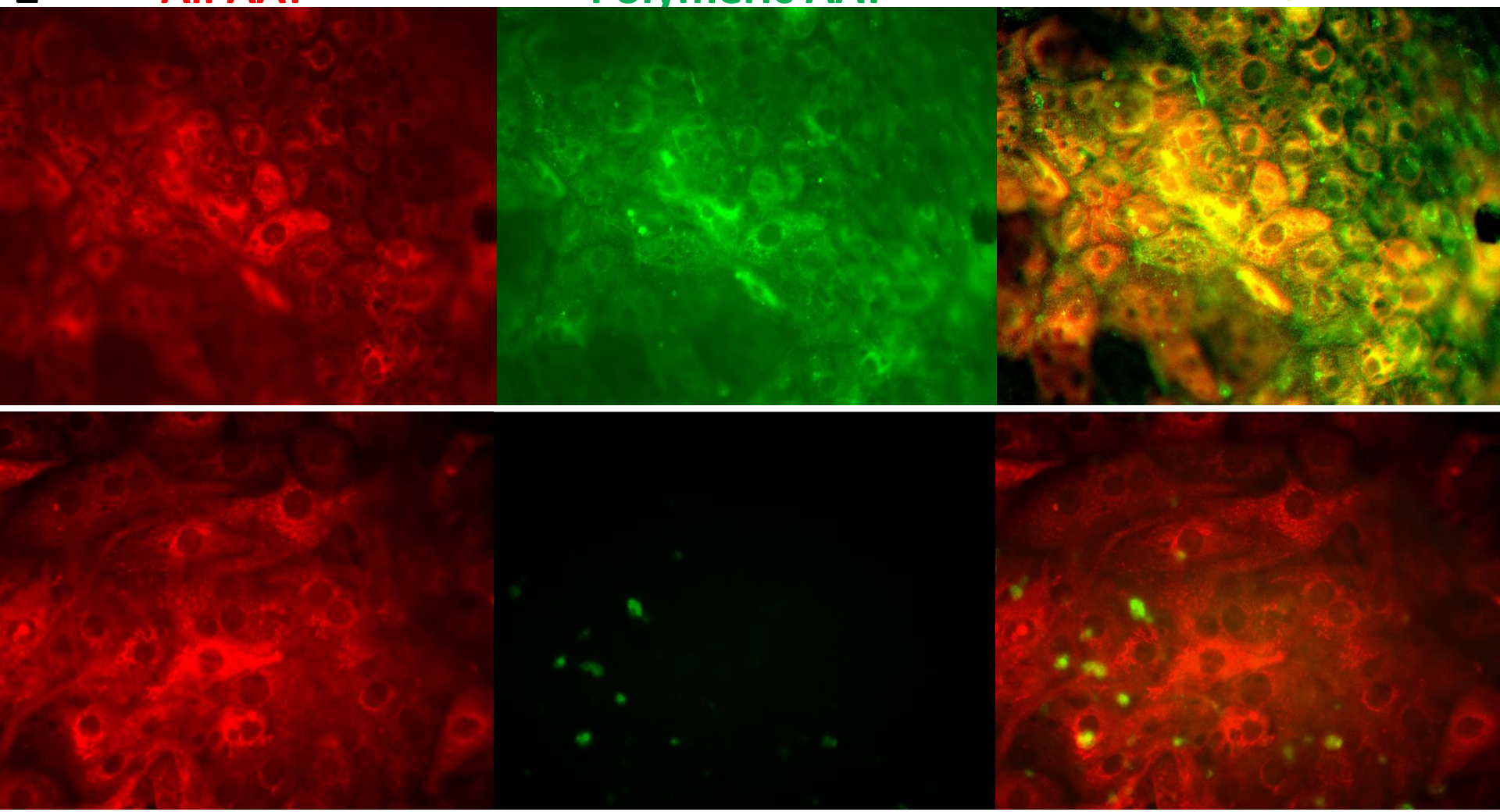
⬇

Assessment in human induced pluripotential stem cells

⬇

Assess in transgenic mouse model of Z α_1-antitrypsin deficiency

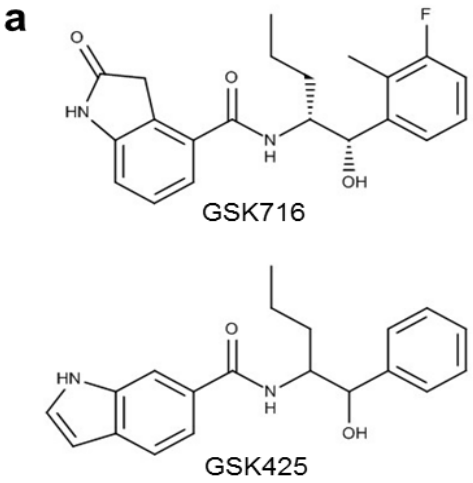
Modello iPSC del deficit di α_1-antitripsina

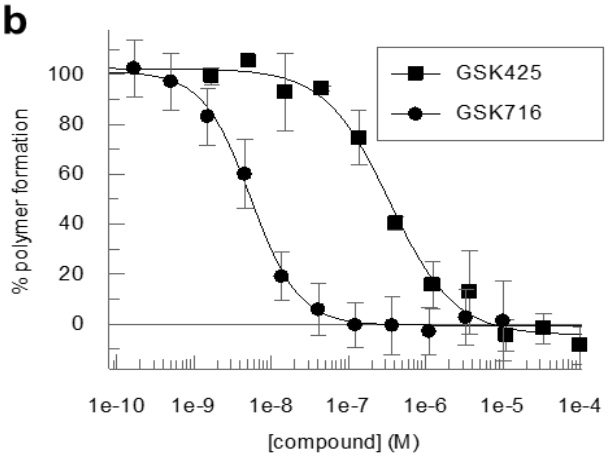


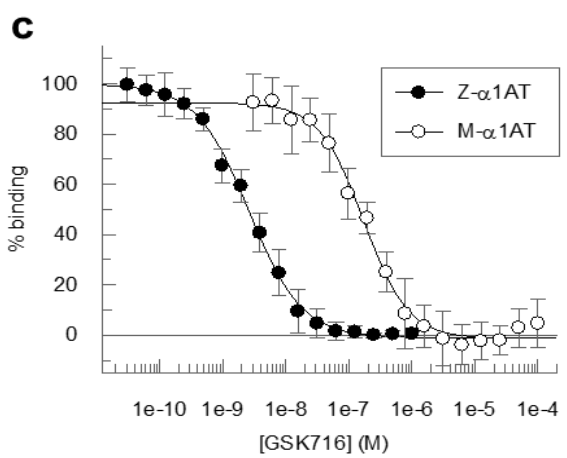
Rashid *et al*, *J Clin Invest* 2010; 120: 3127-3136

Yusa *et al*, *Nature* 2011; 478: 391-394

Composti ‘425 e ‘716

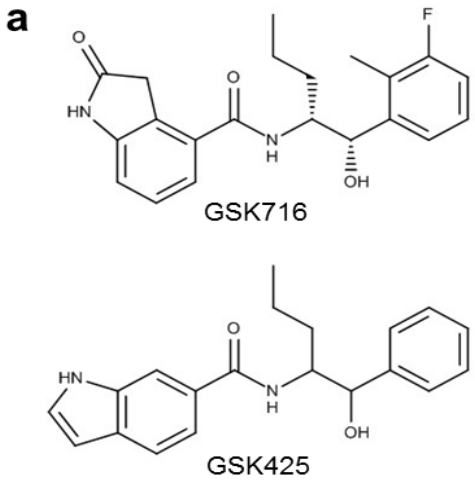


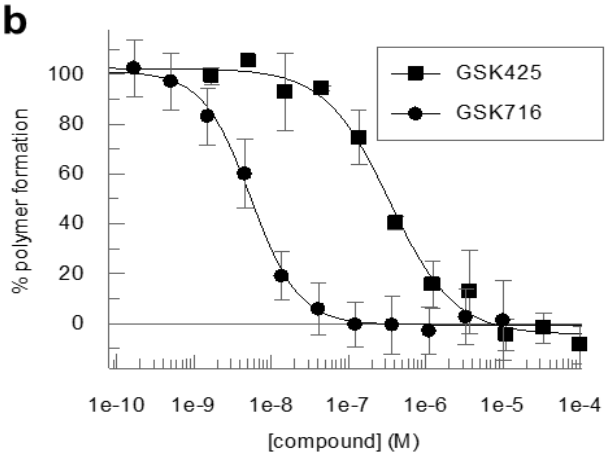


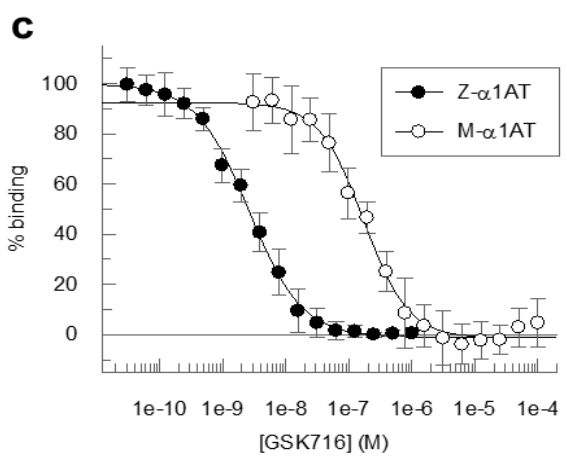


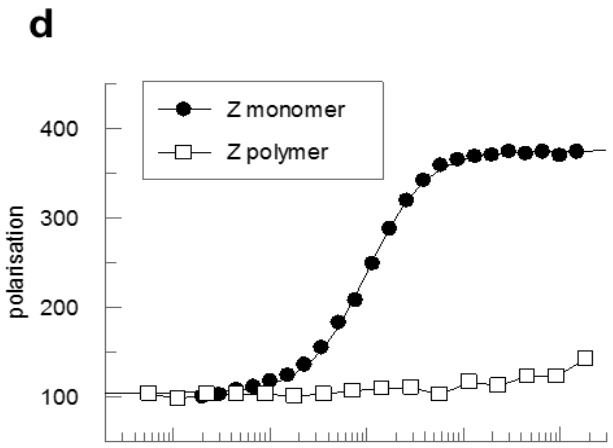
Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

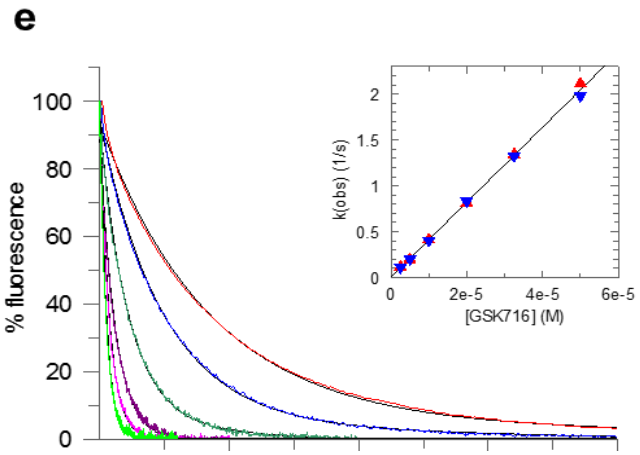
Composti ‘425 e ‘716

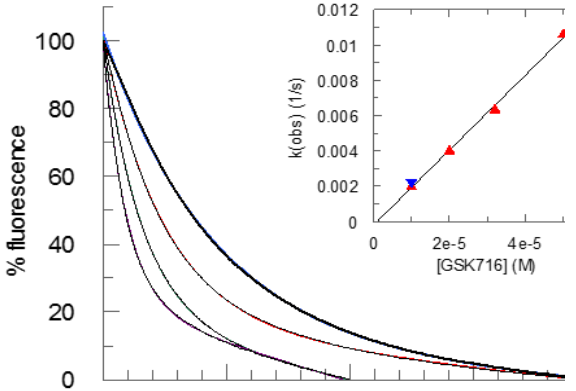










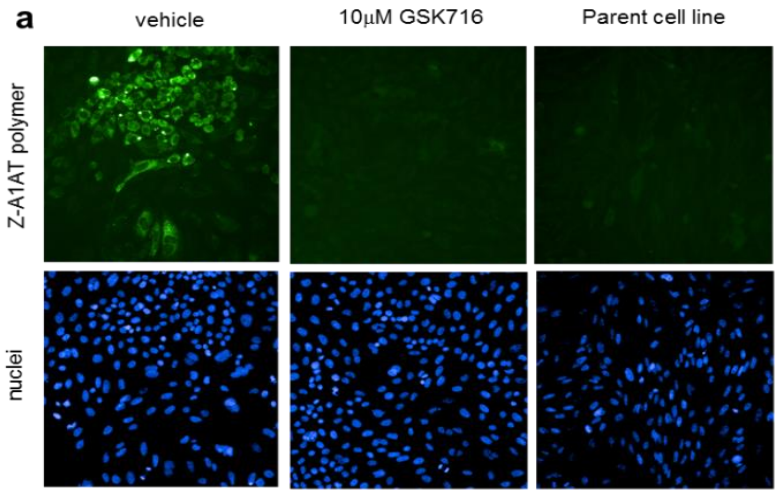


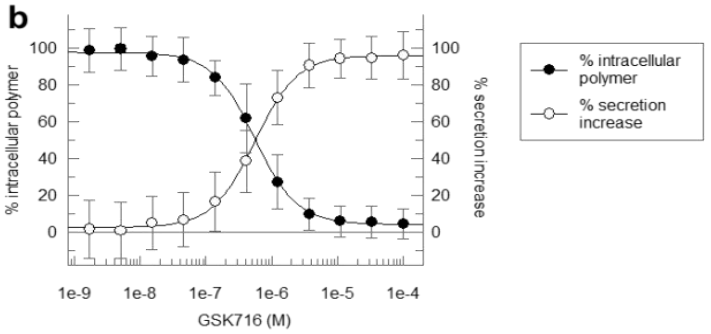
Z AAT $k_{ass}=4.1x10^{4}M^{-1}\ s^{-1}$

M AAT $k_{ass}=2.1x10^{2}M^{-1}\ s^{-1}$

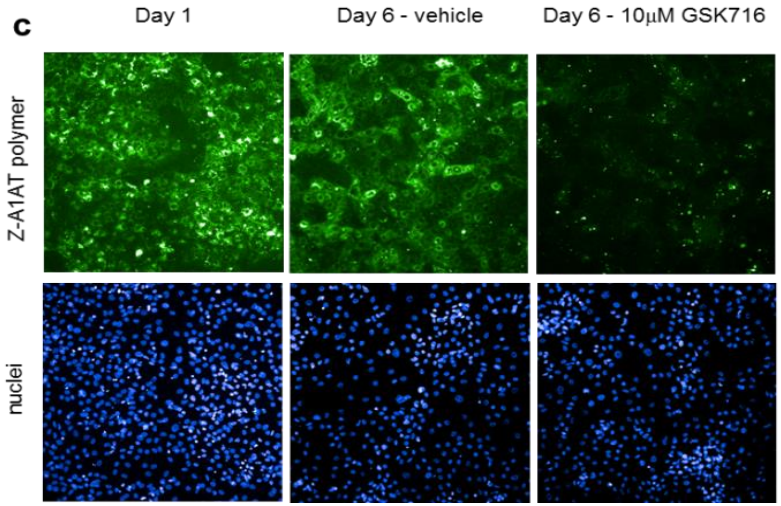
GSK '716 blocca la polimerizzazione in modelli cellulari di malattia

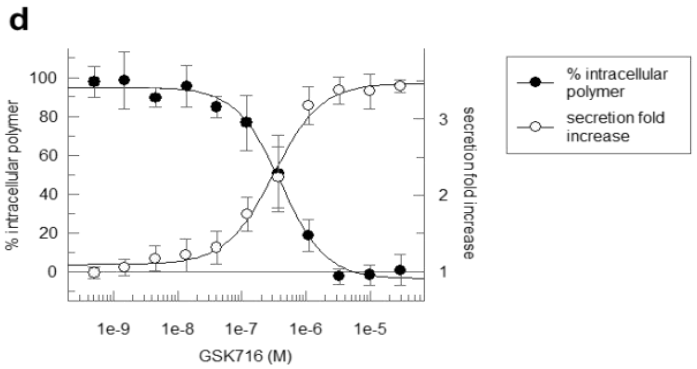
CHO cells





iPSC derived hepatocytes





Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

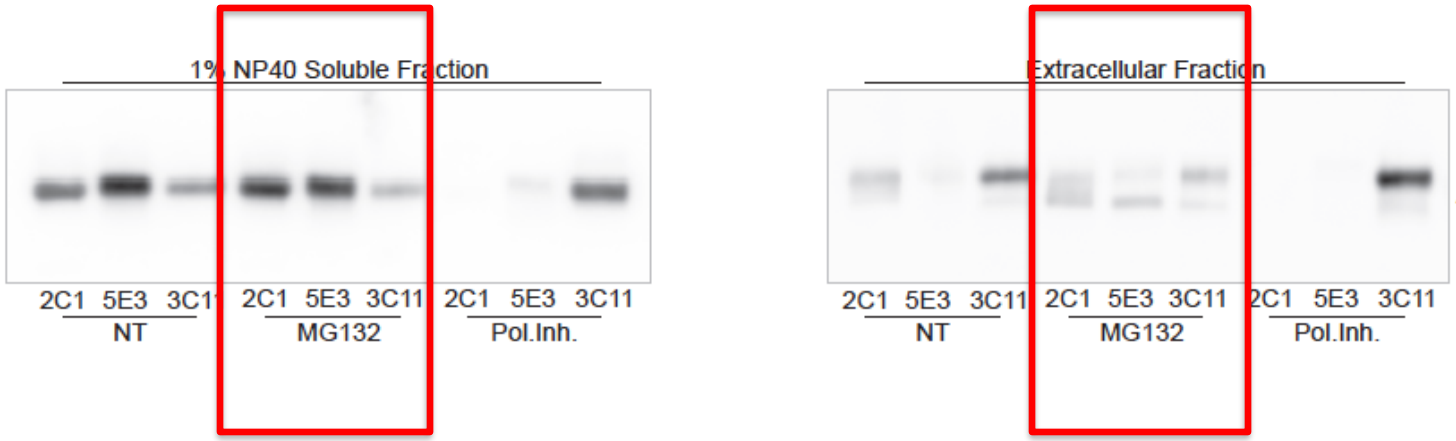
GSK '716 blocca la polimerizzazione in modelli cellulari di malattia

Steady state experiments

Riccardo Ronzoni

Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

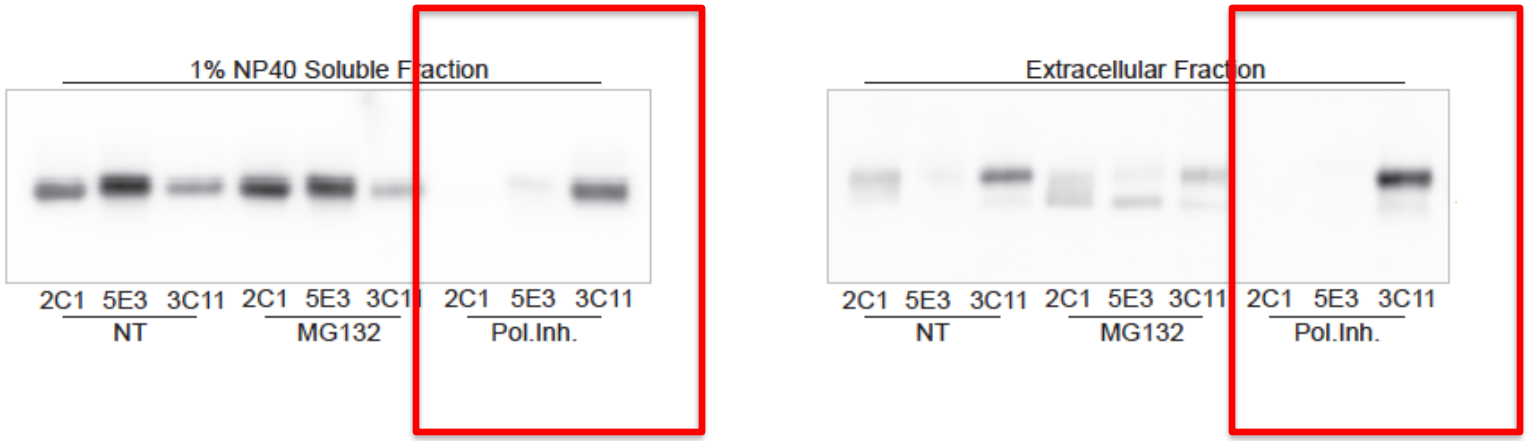
GSK '716 blocca la polimerizzazione in modelli cellulari di malattia



Steady state experiments

Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

GSK '716 blocca la polimerizzazione in modelli cellulari di malattia

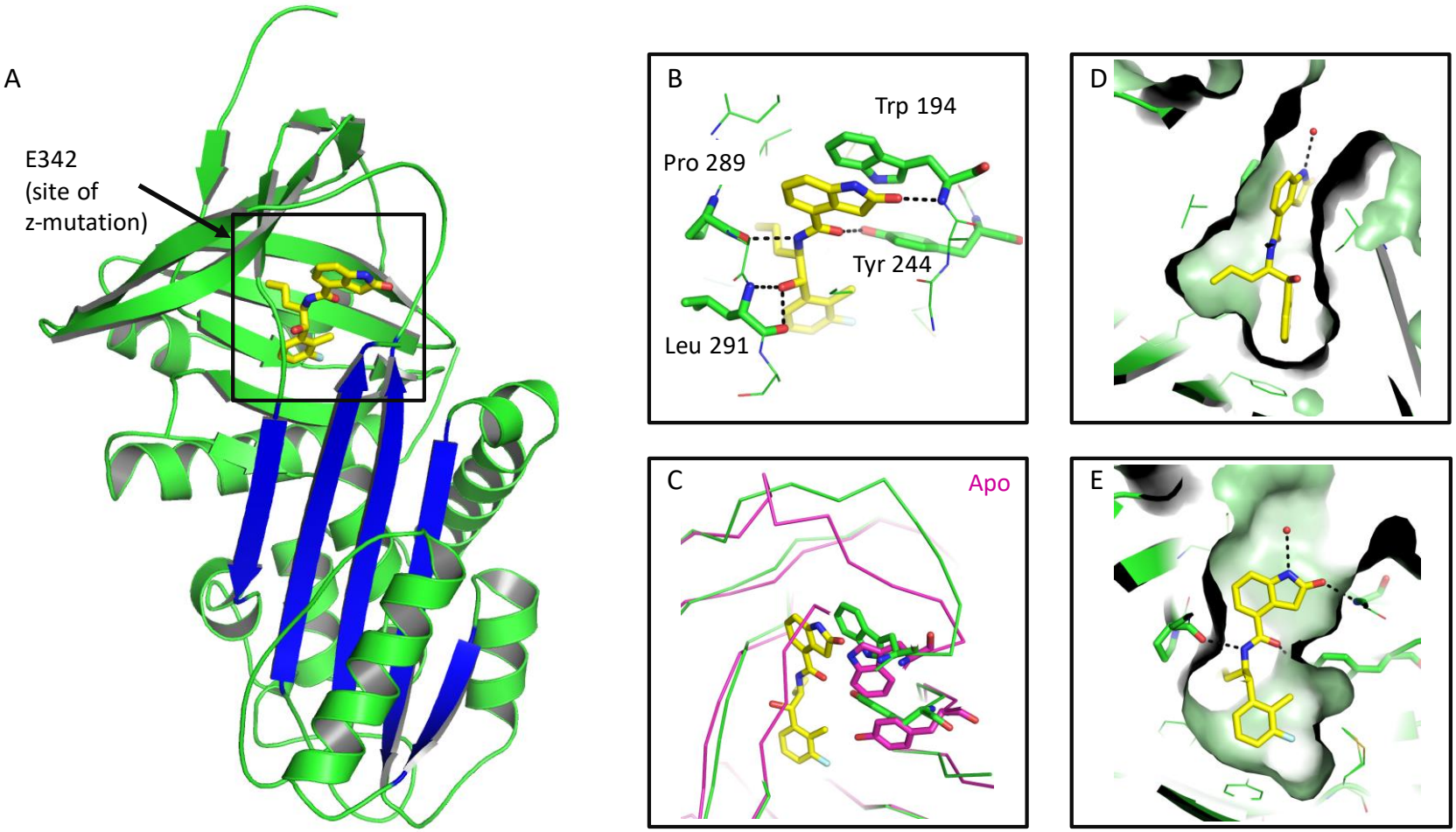


Steady state experiments

Riccardo Ronzoni

Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

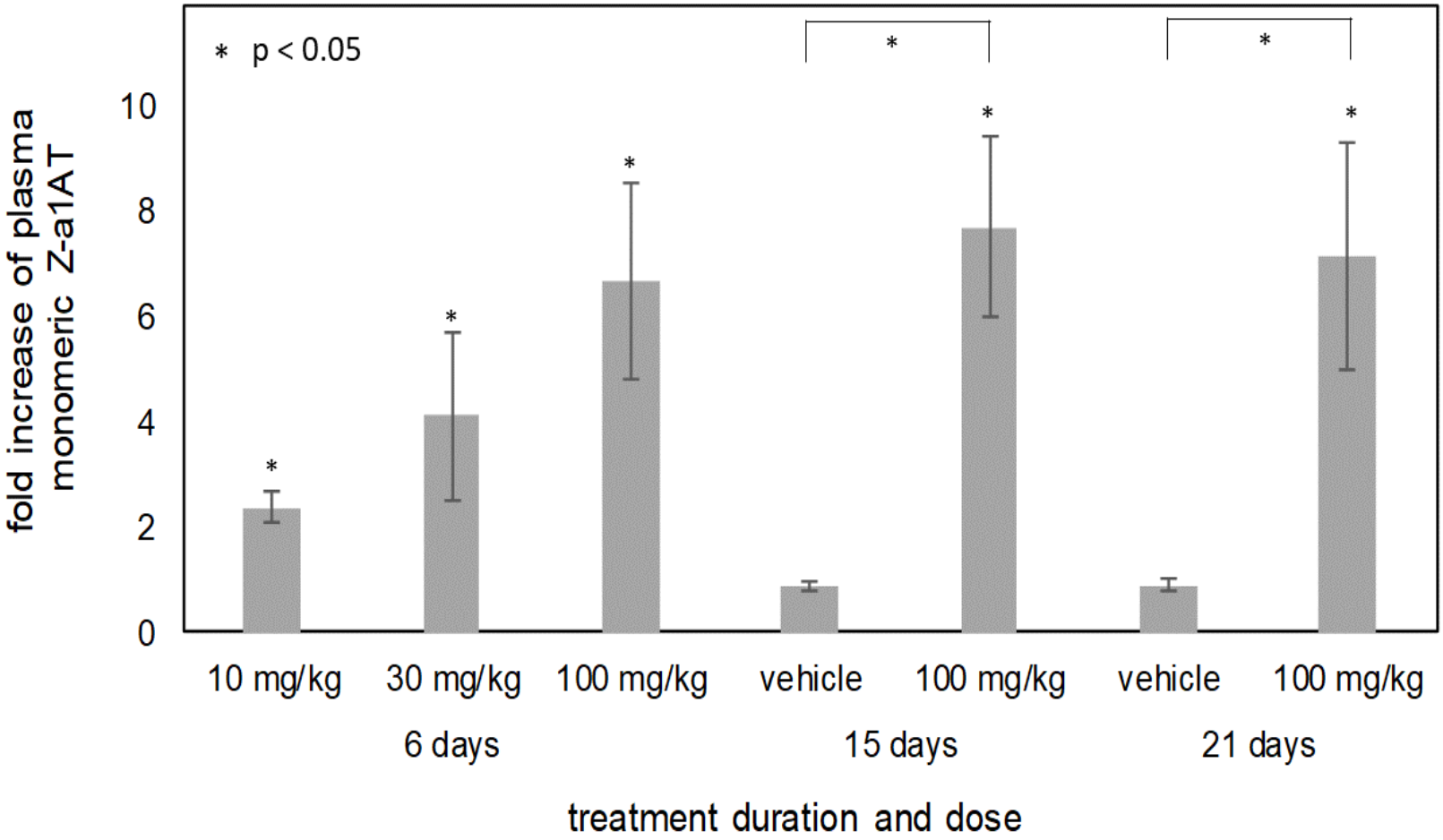
GSK '716 si lega a un nuovo sito di legame criptico



protein-ligand complement of the substituted phenyl and benzoxazolone rings

Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

GSK '716 aumenta la secrezione in un modello murino transgenico



Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

GSK '716 aumenta la secrezione in un modello murino transgenico

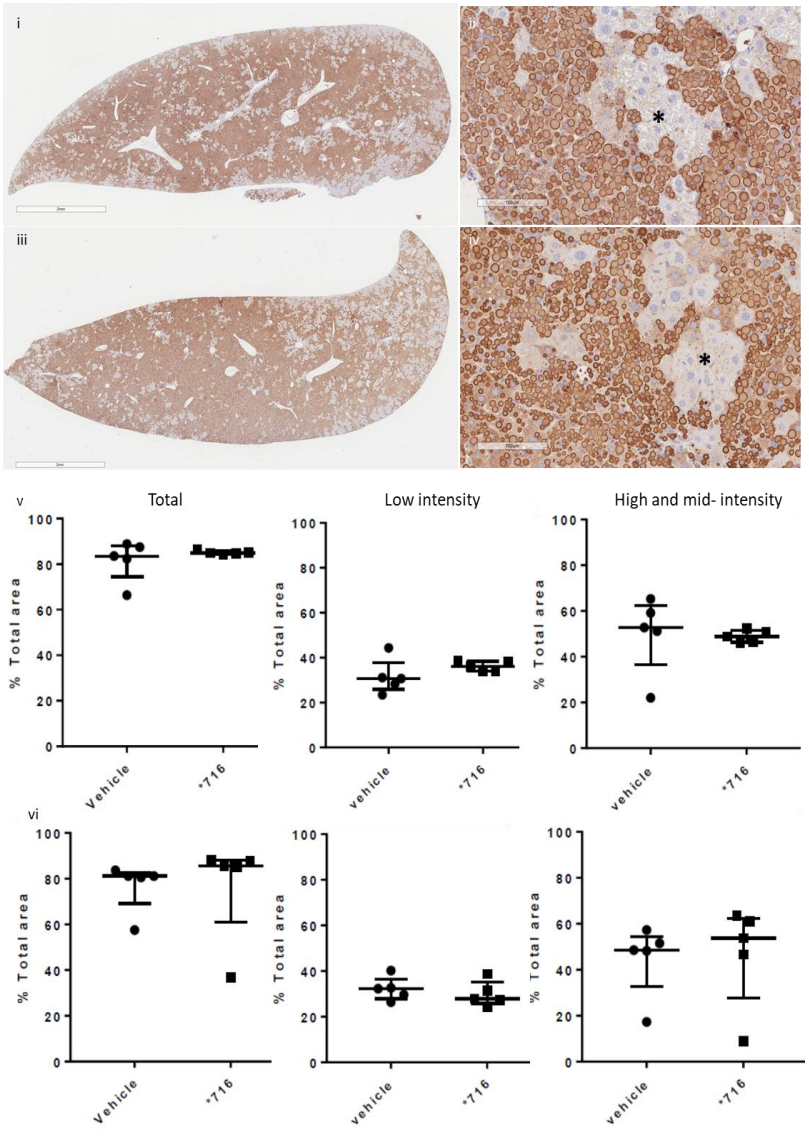
Increase in Z α_1-antitrypsin in the circulation at 10 and 30mg/kg of GSK716 surprising as systemic free drug levels were below the cellular EC50 for secretion for much or all of the dosing period.

Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

GSK716 - nessun effetto sulle inclusioni intraepatiche

Day 15

Day 21



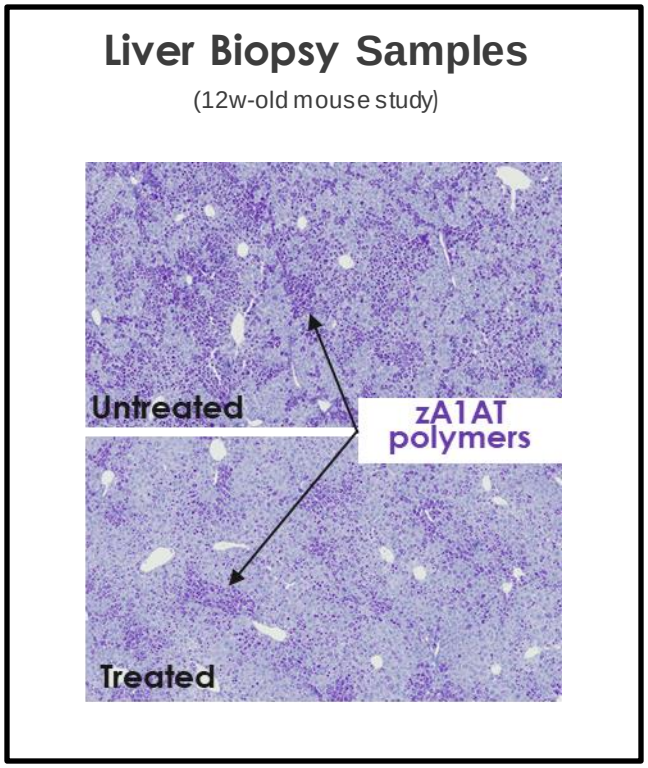
Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

GSK '716 – effetti fuori bersaglio

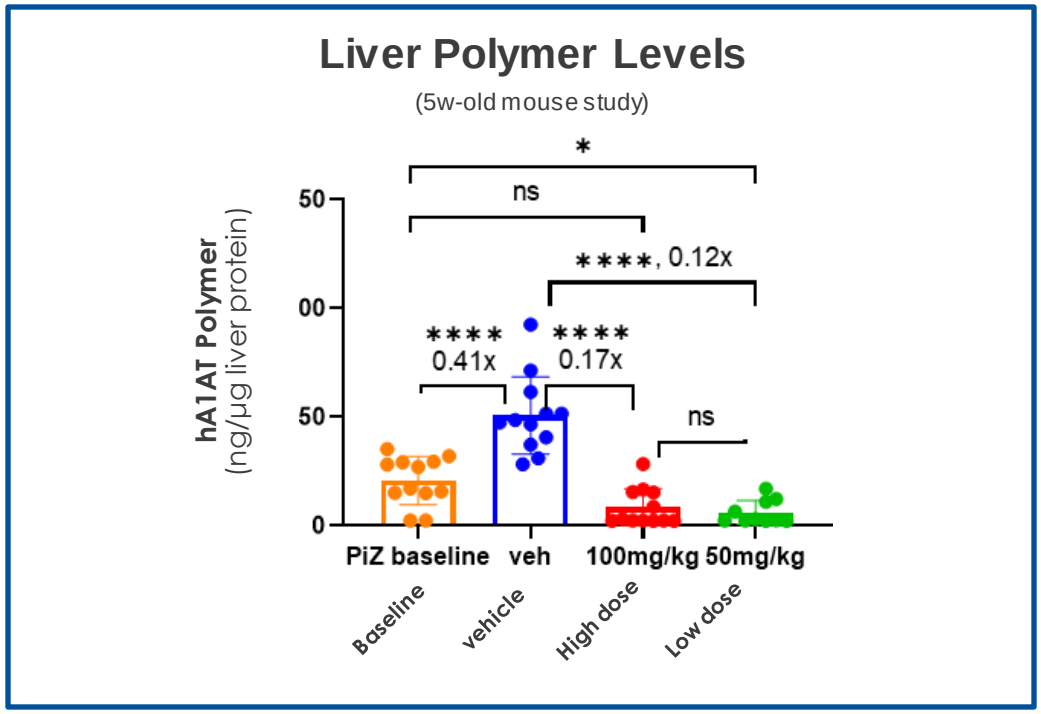
No off target effects in a panel of 85 assays considered predictive of known safety liabilities that precluded further development of GSK716

Lomas *et al*, *EMBO Mol. Med.* 2021; 13(3):e13167

BMN 349 ha ridotto significativamente l'accumulo di polimeri nei modelli murini



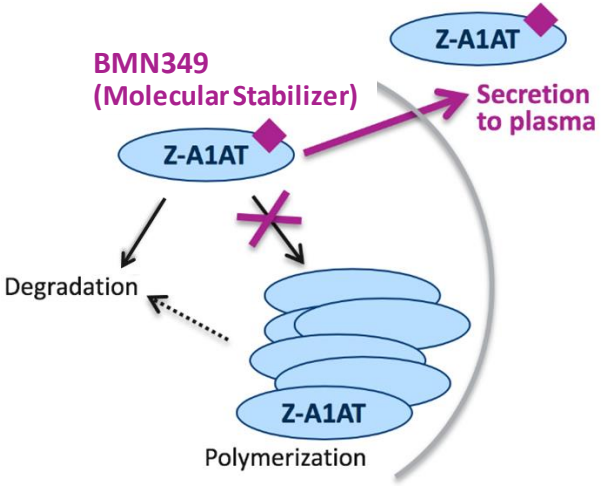
Treatment for 1 month

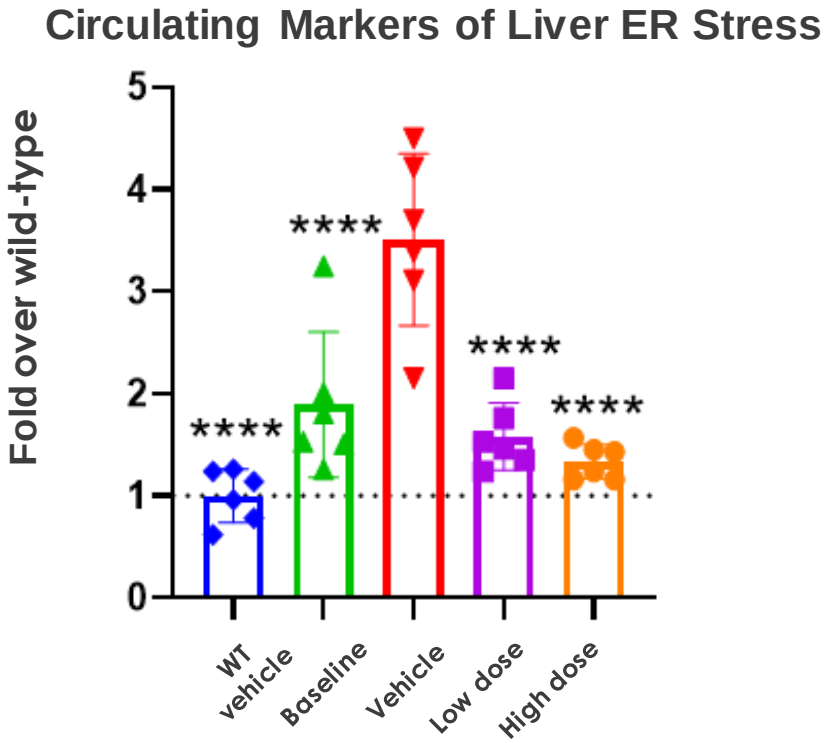


Mouse model recapitulates human liver disease and shows

- **Decreased aggregation in the liver**
- **Increased secretion of nonfunctional zA1AT monomer**
- **Regeneration of progressive liver disease**

BMN 349 ha ridotto significativamente i marcatori di stress epatico nei modelli murini



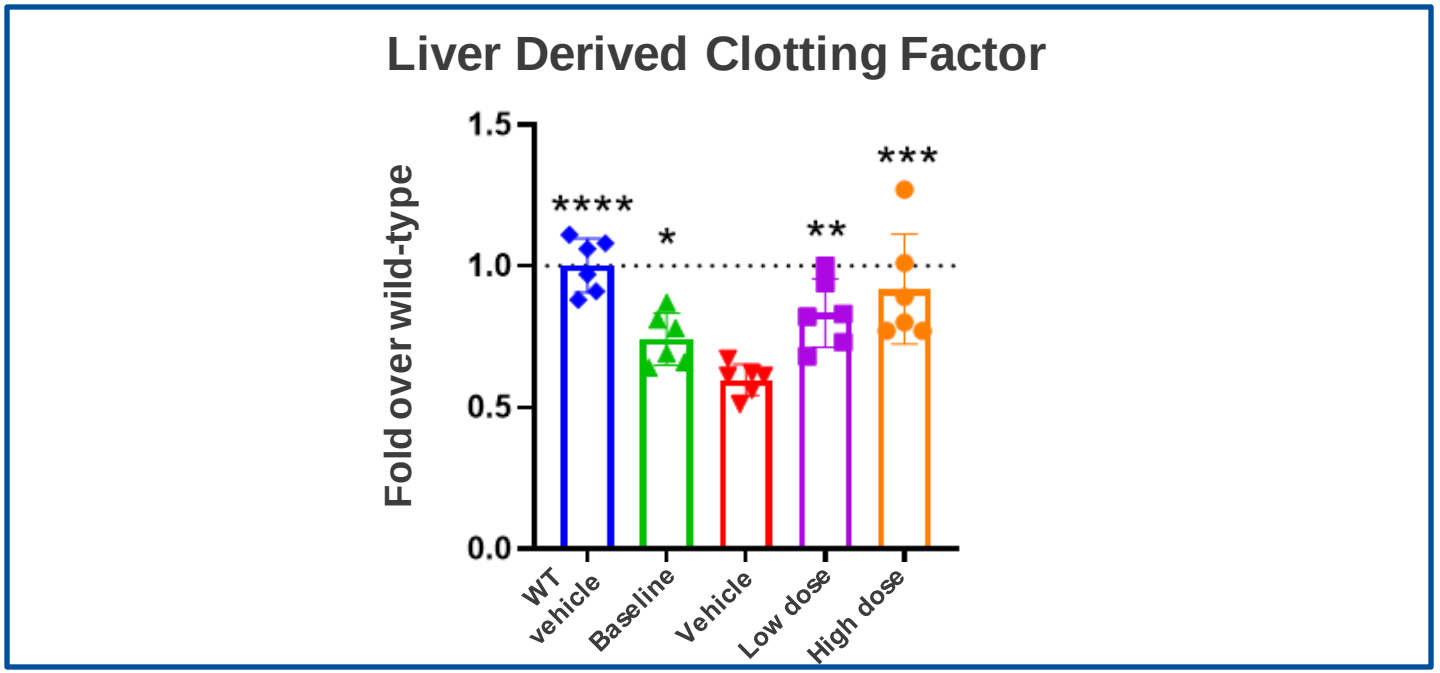


Mouse model recapitulates human liver disease and shows

- **Decreased aggregation in the liver**
- **Reduction in markers of endoplasmic reticulum stress, among the pathologic cell responses to polymers**
- **Rapid onset of cell response to therapy**

Biomarin R&D day 2021

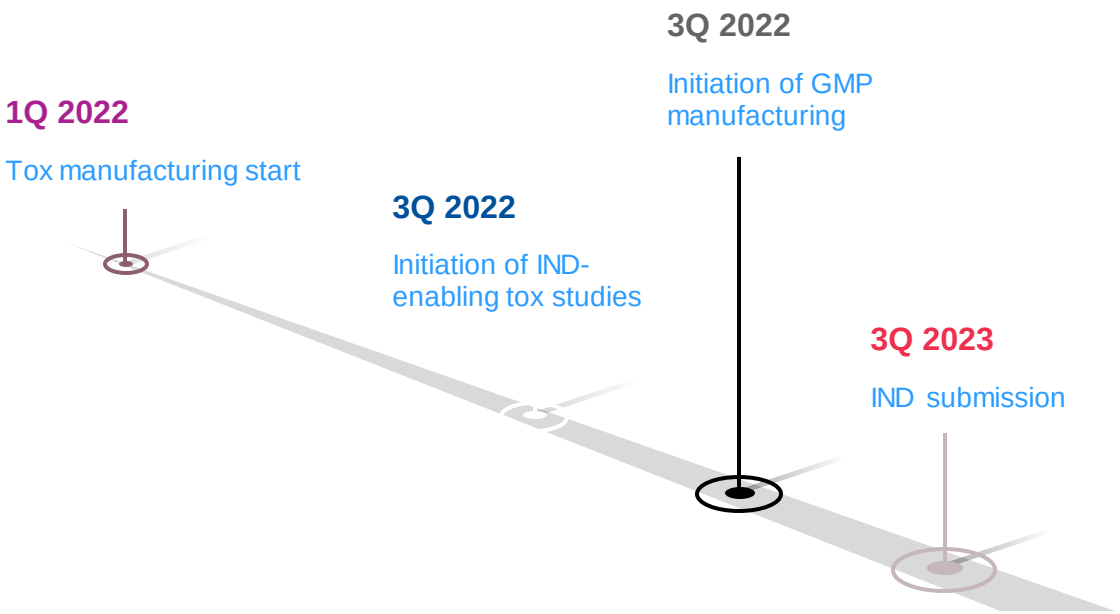
BMN 349 ripristina la normale funzione sintetica nei modelli murini



Mouse model recapitulates human liver disease and shows

- **Decreased aggregation in the liver**
- **Reversal of impaired hepatocyte function**
- **Rapid response of normal important hepatocyte functions to treatment**

BMN 349 -piani



Piccole molecole: benefici

Titratable dosing

Effective in MZ heterozygotes

Conclusioni

1. α_1-antitrypsin deficiency results from a domain swap polymerisation

2. This is a general mechanism that affects other serine proteinase inhibitors (the serpinopathies)

3. Blocking polymerisation provides a novel strategy to prevent disease

Ringraziamenti

Medical Research Council (UK)

EPSRC

Wellcome Trust

Alpha-one foundation (US)

GlaxoSmithKline

National Institute for Health Research

Biomarin

Ringraziamenti

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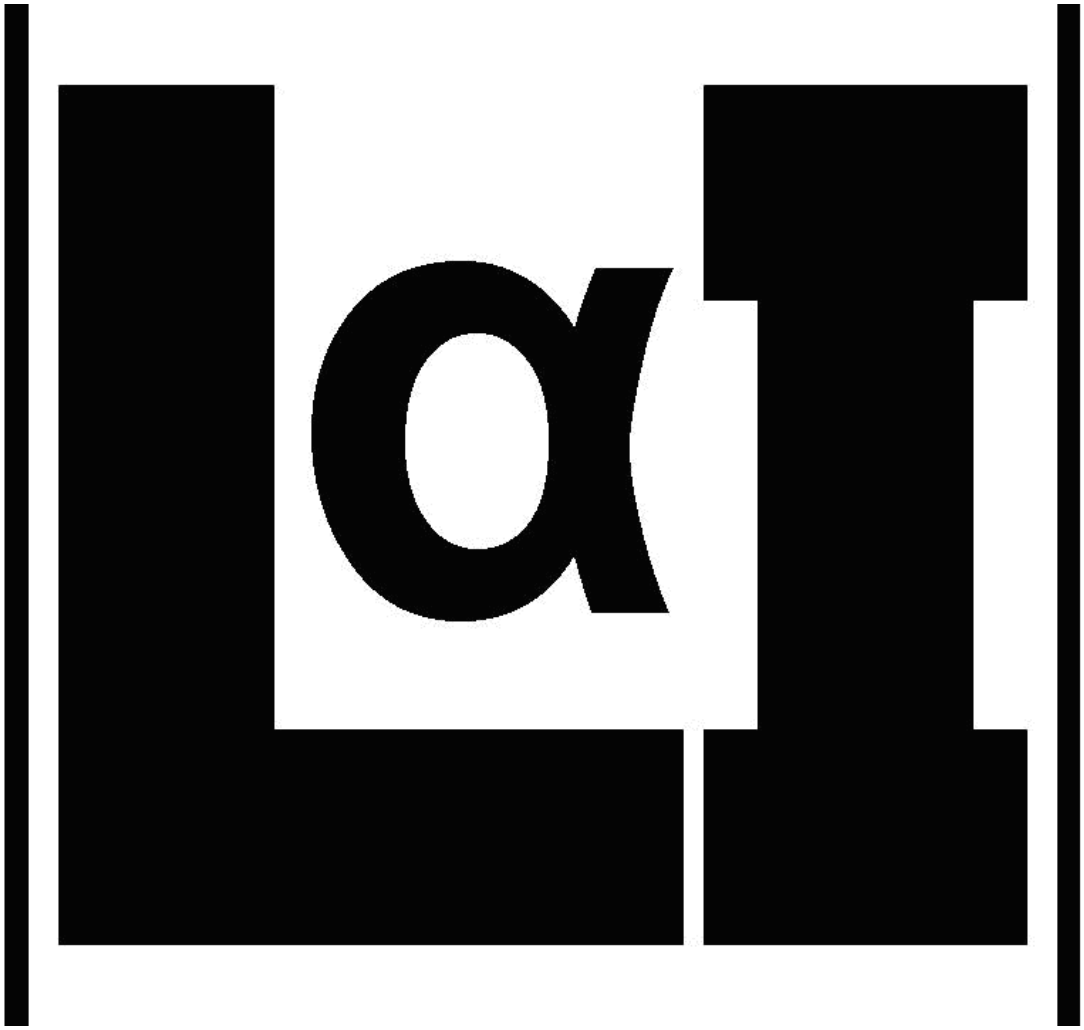

The London α_1-antitrypsin deficiency service at the Royal Free Hospital

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