

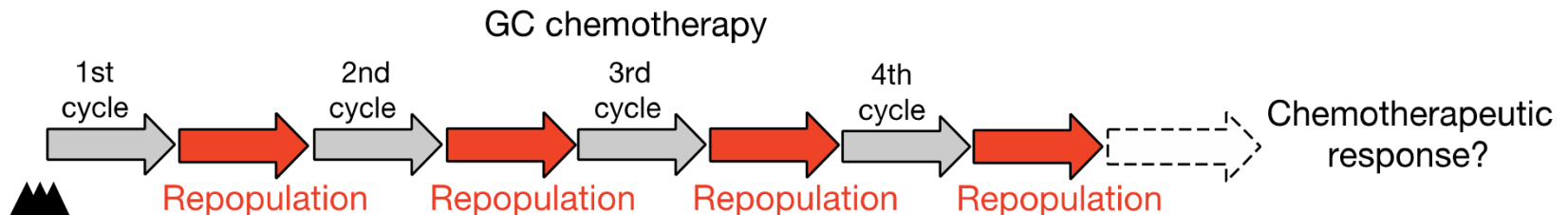
Blocking PGE₂ – induced tumour repopulation abrogates bladder cancer chemoresistance

Antonina V. Kurtova et al.

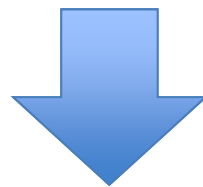
Nature. 2015 Jan 8

Überblick

Im Mittelpunkt aktueller Studien:



Resistenz gegenüber Chemotherapie (70% bei
Harnblasen CA)



Krebsstammzellen (CSC) mit Überlebensvorteil

Überblick

In dieser Studie:

Resistenz von CSCs bei Blasenkrebs

1. Verstärkte Zellaktivität von CSCs nach chemotherapieinduzierten Schäden (*Ähnlichkeit zur Wundreparatur*)
2. Zusammenhang
PGE₂-Ausschüttung → CSCs Anhäufung
3. Dämpfung der Resistenz durch PGE₂-Hemmung (celecoxib, in vivo)

Harnblasen-CA

- Cytokeratin 14-Zellen (**CK14**)– primitivster Differenzierungsgrad – „*CSCs bei Harnblasen-CA*“
- Patienten mit viel CK14 – schlechteres Überleben nach Chemotherapie

a

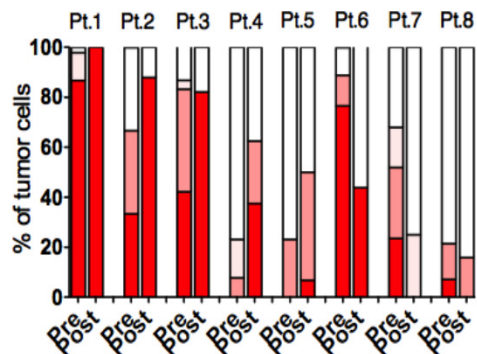
Pt	Age	M / F	NAC	Pre-NAC			Post-NAC			Follow up (months)	NED	DOD	
				Clinical T stage	Grade	Histology	Pathological T stage	Grade	Histology				N-stage
1	48	F	GC	T4a	HG	TCC ¹	T4a	HG	TCC	N3	11	-	+
2	67	F	MVAC	T3	HG	TCC	T3b	HG	TCC	N0	12	-	+
3^{PDX}	49	M	GC	T3b	HG	TCC¹	T3b	HG	TCC⁴	N0	17	-	+
4	49	F	GC	T4	HG	TCC	T4a	HG	TCC ¹	N2	31	-	+
5	58	M	GC	T3	HG	TCC	T2b	HG	TCC	N3	19	-	+
6	70	M	GC	T4a	HG	TCC	T3b	HG	TCC	N2	28	-	+
7	72	M	GC	T3	HG	TCC ²	T0	N/A	N/A	N0	15	-	+
8	81	M	EC	T3	HG	TCC ³	T3b	HG	TCC ³	N1	9	-	+
9	59	F	GC	T3b	HG	TCC	T2a	HG	TCC	N3	28	-	-
10	65	M	GC	T3b	HG	TCC	T0	N/A	N/A	N0	21	+	-
11	70	M	GC	T4a	HG	TCC	T4a	HG	TCC	N0	43	+	-
12	72	M	GC	T3a	HG	TCC	T0	N/A	N/A	N0	28	+	-
13	61	M	GC	T4a	HG	TCC	T1	HG	TCC	N0	52	+	-
14	57	M	GC	T4a	HG	TCC	T3a	HG	TCC	N0	27	+	+
15	74	M	GC	T2b	HG	TCC	Tis	HG	TCC	N0	31	+	-

!

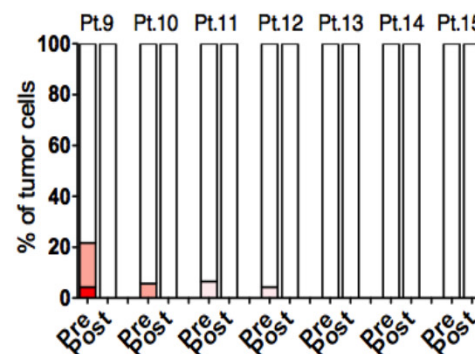
Pt – patient; NAC – neoadjuvant chemotherapy; NED – no evidence of disease; DOD – dead of disease; GC – gemcitabine+cisplatin; MVAC – methotrexate, vinblastine, doxorubicin, cisplatin; EC – etoposide+carboplatin; HG – high grade; TCC – transitional cell carcinoma (¹-with squamous differentiation, ²- with squamous and glandular differentiation, ³-with neuroendocrine features, ⁴-with squamous and sarcomatoid differentiation)

b

CK14 Enrichment/Persistence

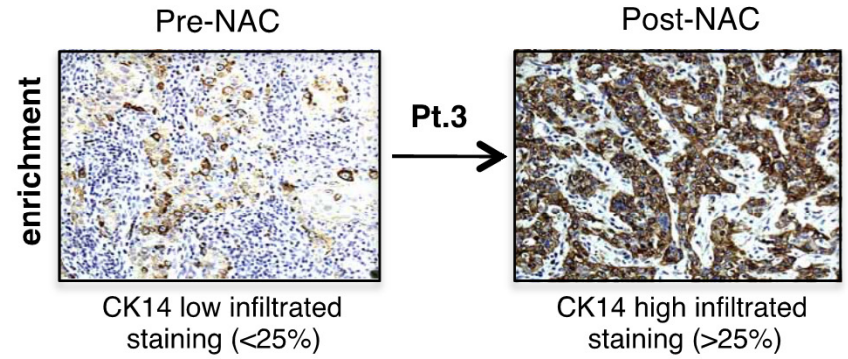
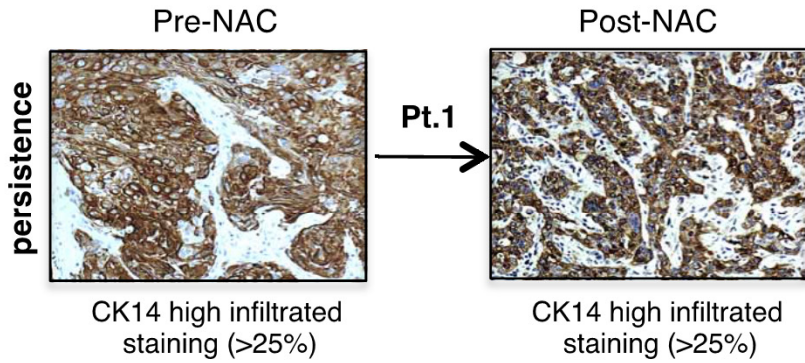


CK14 Resolution

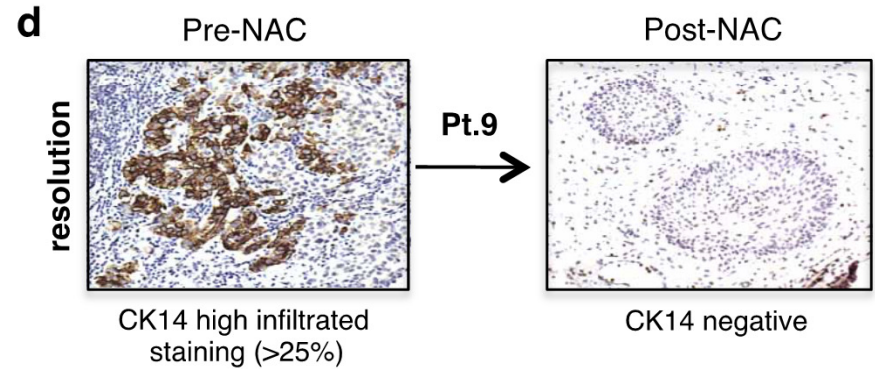
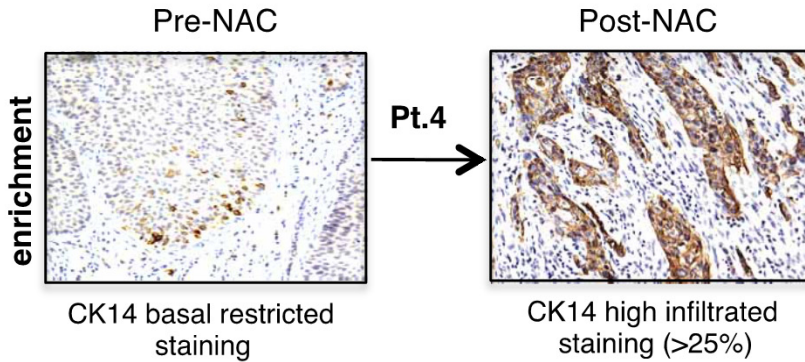


CK14 negative
 CK14 basal
 CK14 low infiltr
 CK14 high infiltr

C CK14 enrichment/persistence

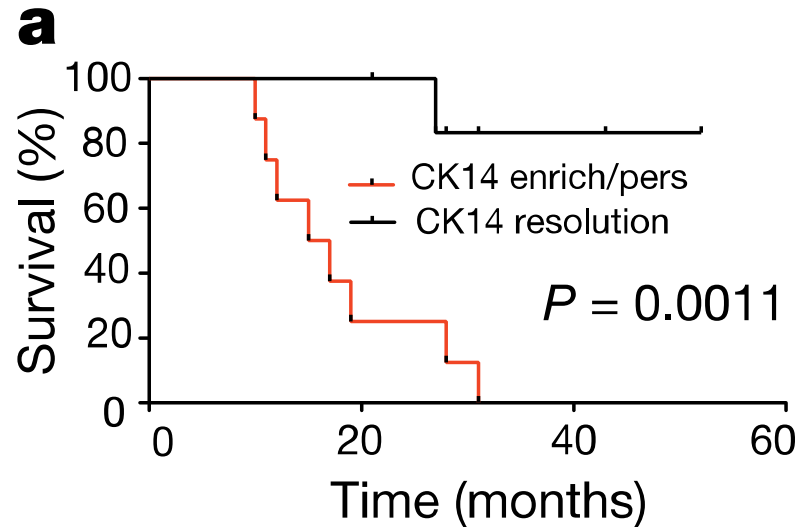


CK14 resolution

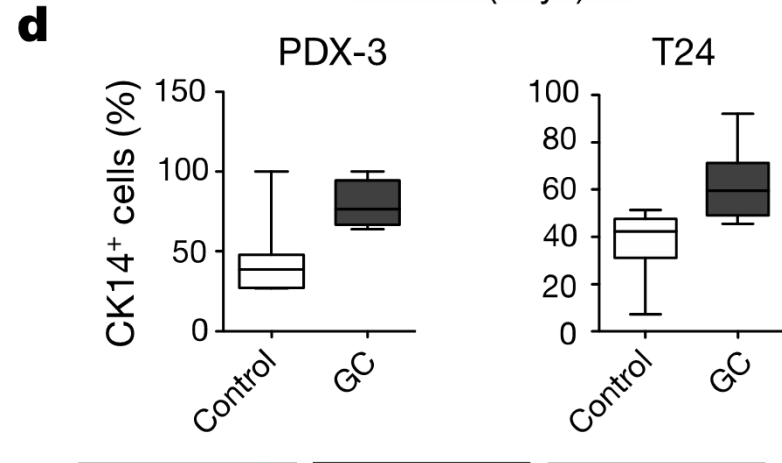
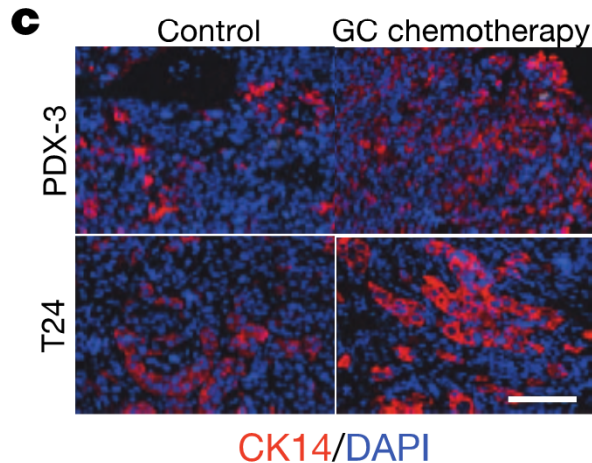
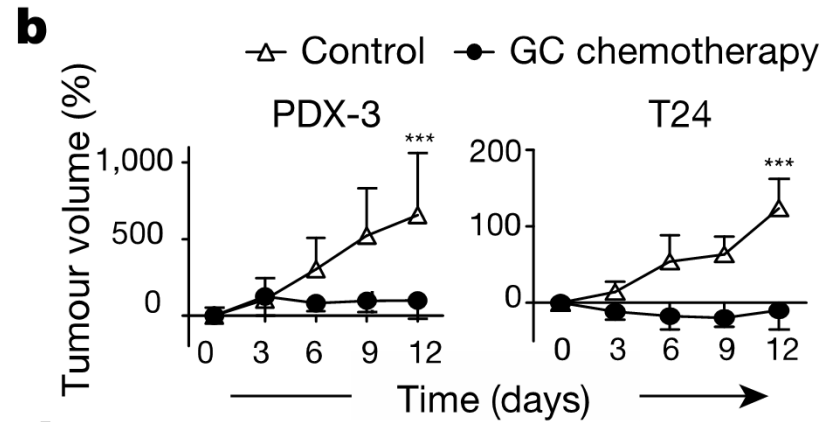
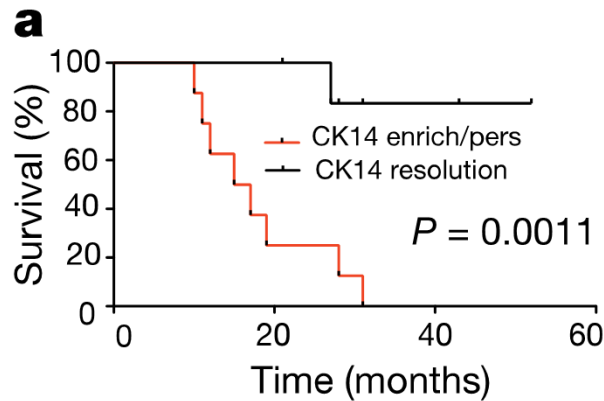


Überblick

- Viel CK14 → Survival ↓

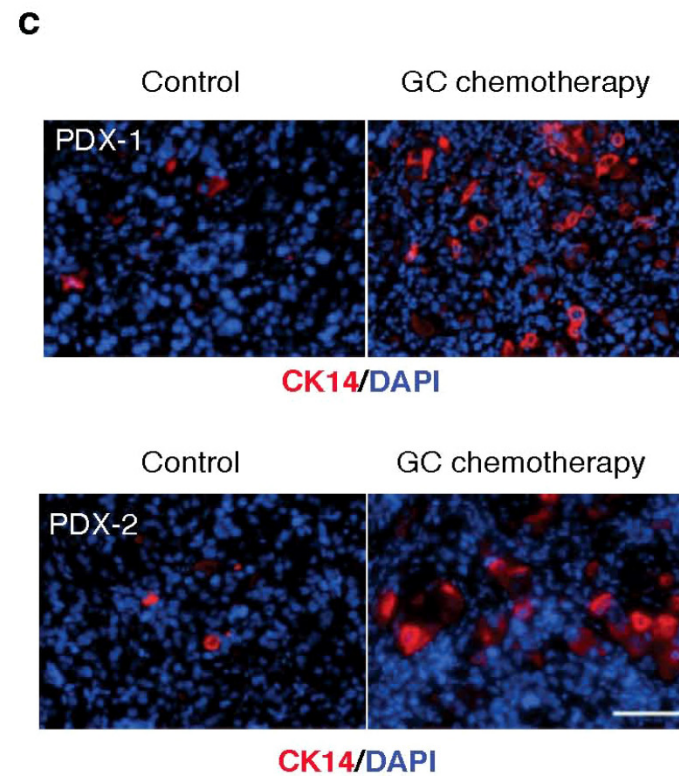
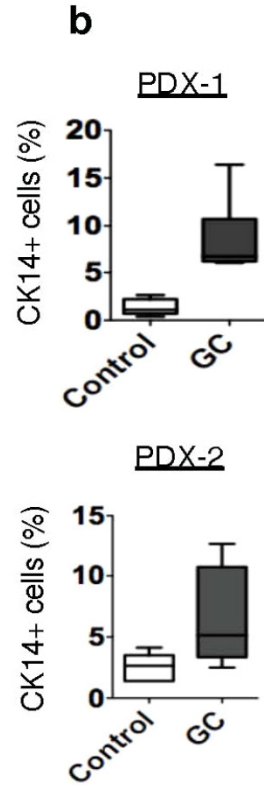
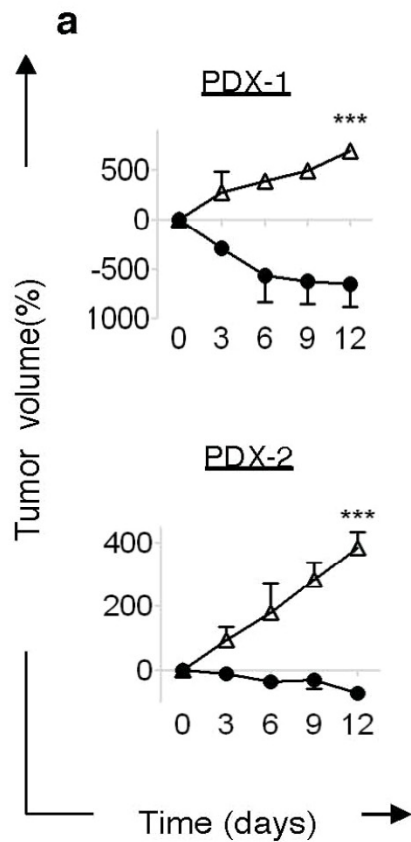


Gemcitabin und Cisplatin (GC)



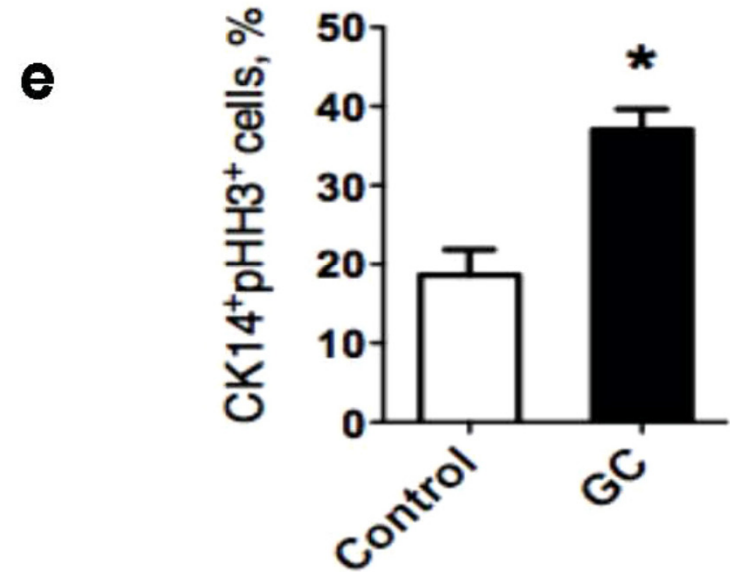
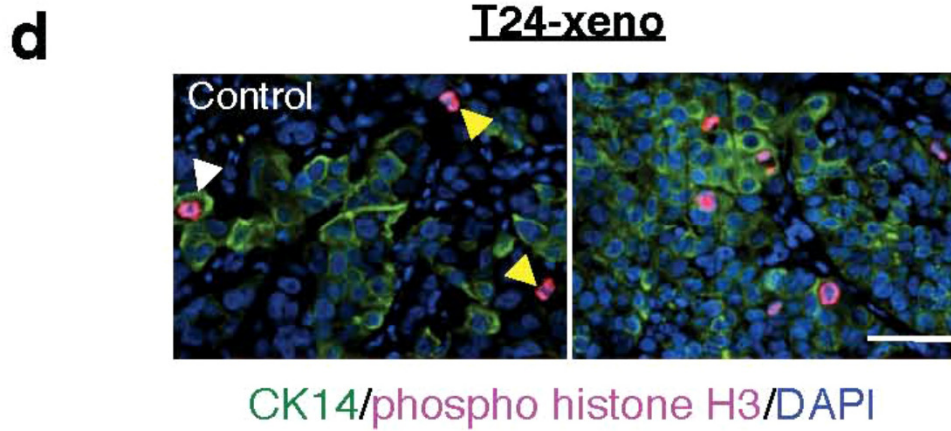
Chemotherapie Gemcitabin und cisplatin (GC)

LETTER RESEARCH



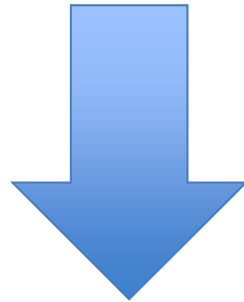
- Nach Chemotherapie:
 - Tumorgröße nimmt ab
 - Ck14+-Zellen reichern sich an

Proliferation



Im Überblick

- erhöhte Proliferation während CK14Anreicherung nach Chemotherapie



Konventioneller Gedanke	Neuer Gedanke
Chemoresistente Tumorzellen werden durch Chemotherapie bevorzugt (Selektion)	„Aktive Prozess/Antwort“ durch Proliferation

Isolierung von CK14+ Zellen

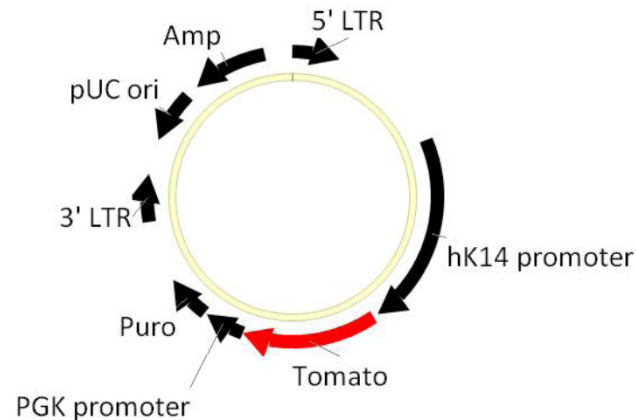
Ziel → Isolation von CK14+ Zellen mittels
Fluoreszenz-activated-cell-sorting (FACS)

Promotorloser, lentiviraler **Vektor** mit **tdTomato**

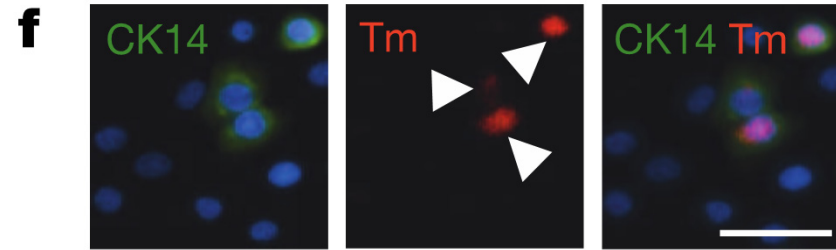
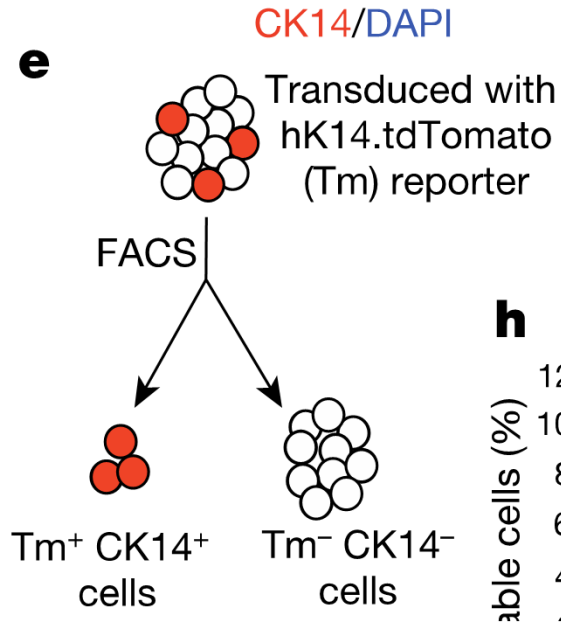
+

Promotorregion **KRT14**

a



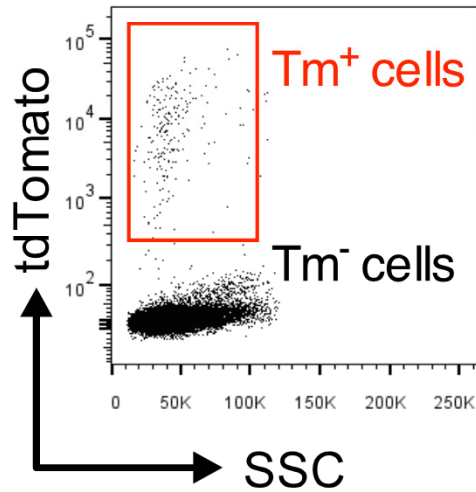
Isolierung von CK14+ Zellen



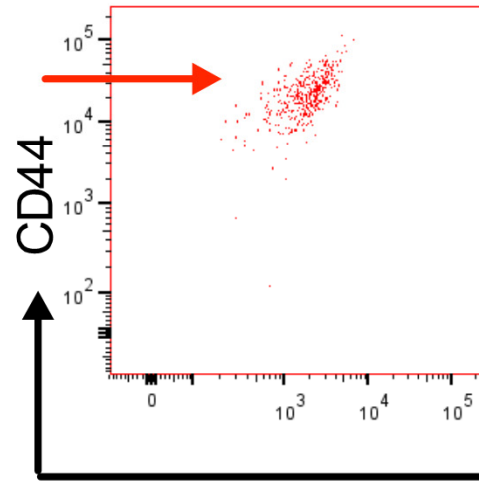
„Tm+ CK14 Zellen“

Isolierung von CK14+ Zellen

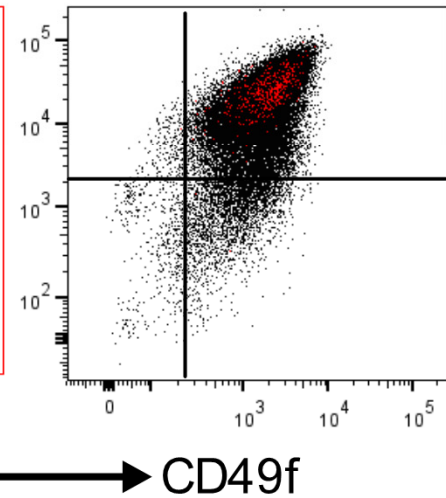
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c

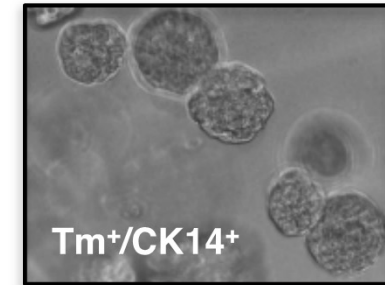
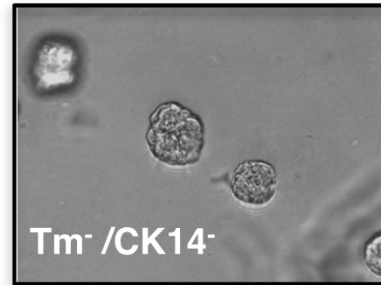
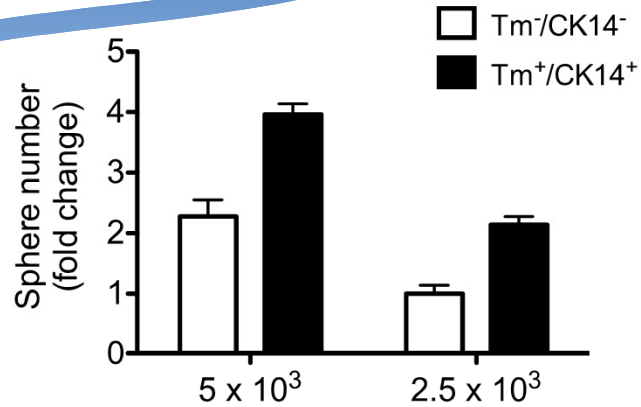


d



Isolierung von CK14+ Zellen

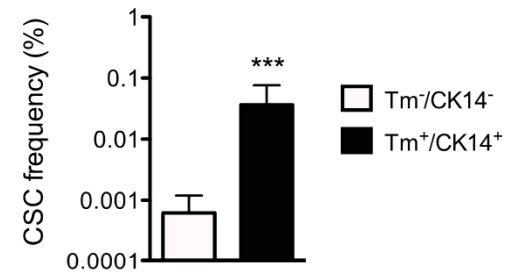
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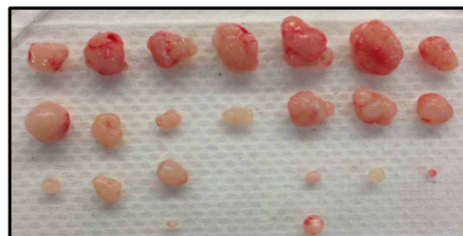
f

Xenograft engrafted

Cells	Tm+/CK14+	Tm-/CK14-
5000	15 / 15	13 / 15
500	7 / 7	5 / 7
50	6 / 7	1 / 7
10	2 / 7	0 / 7



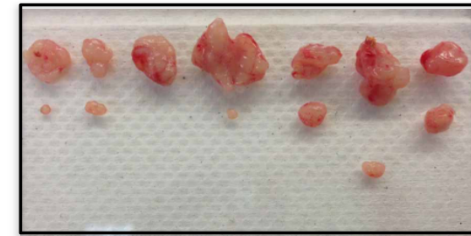
Tm+/CK14+ cancer cells



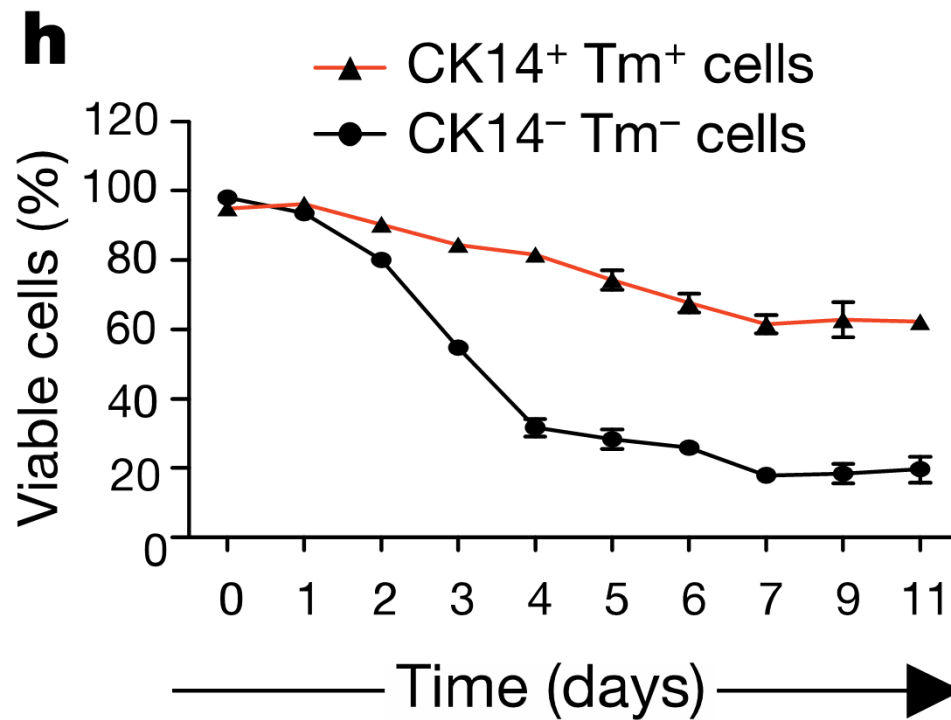
Cell number

5000
500
50
10

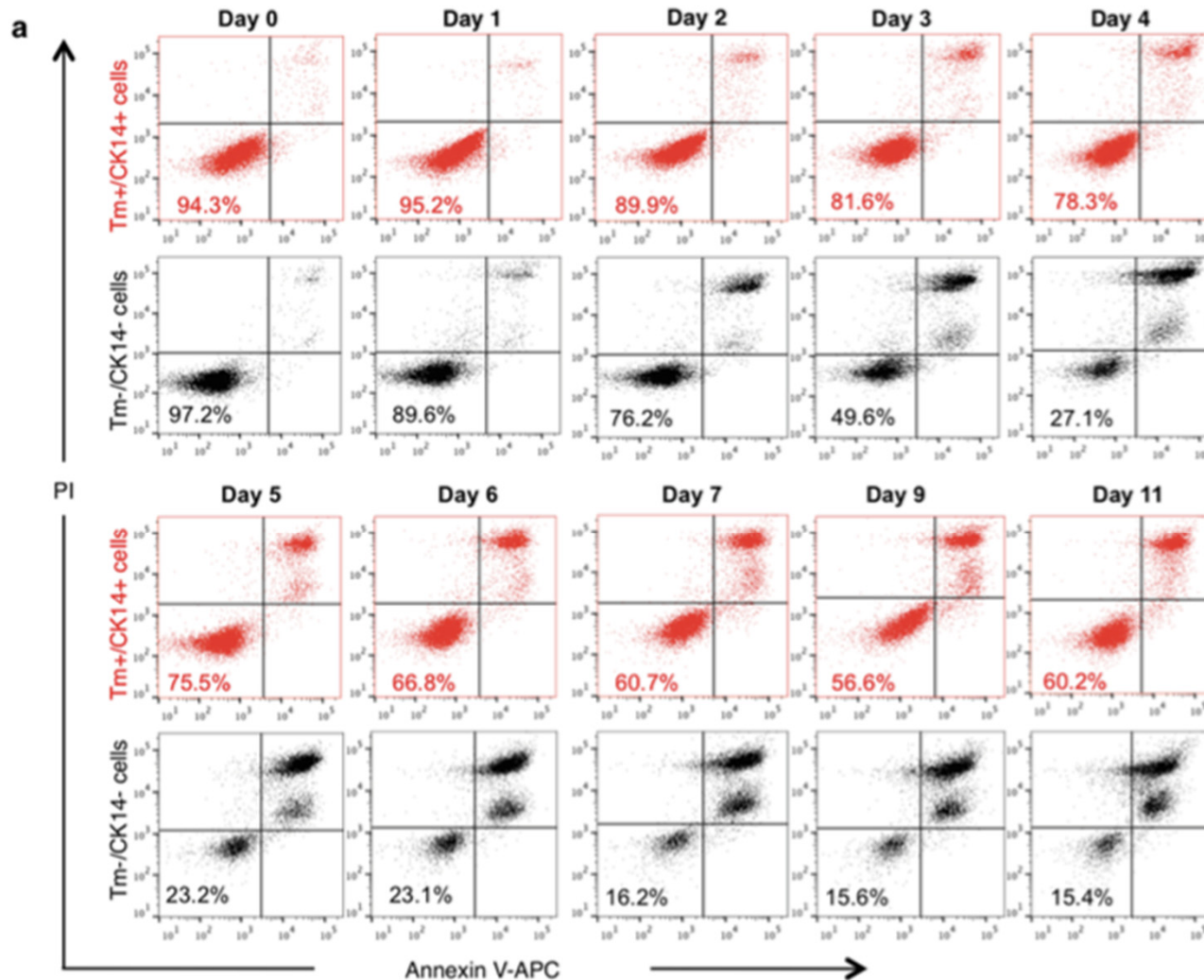
Tm-/CK14- cancer cells



Tm+CK14+ Zellen unter Chemotherapie



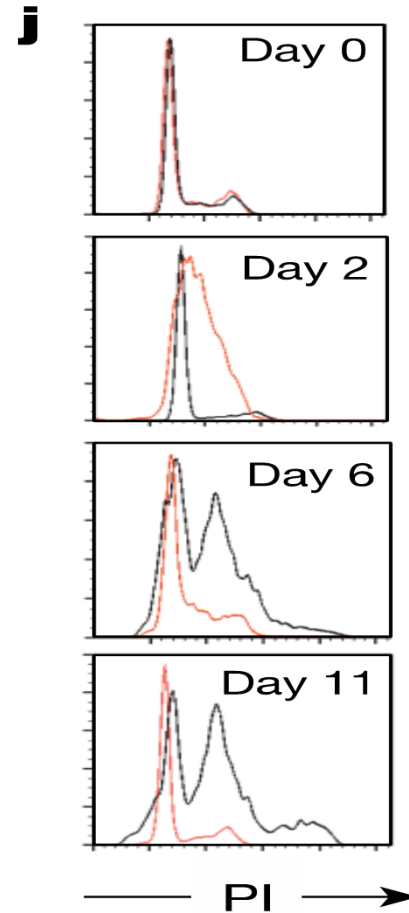
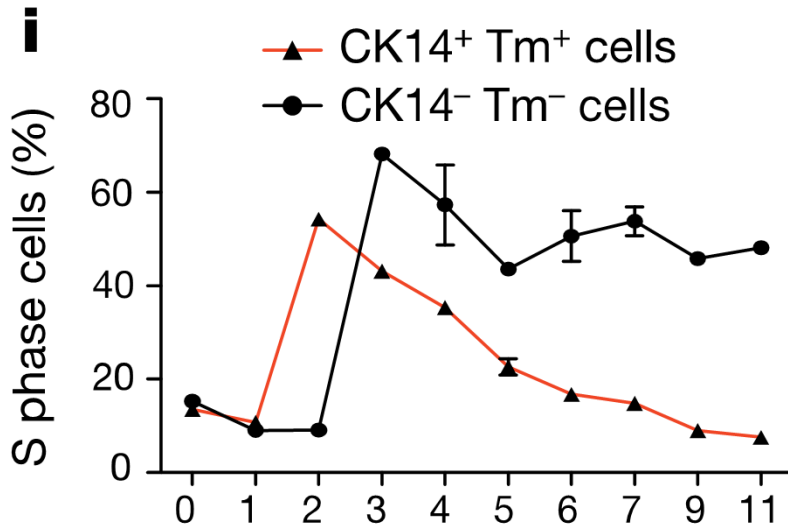
Tm+CK14+ Zellen unter Chemotherapie



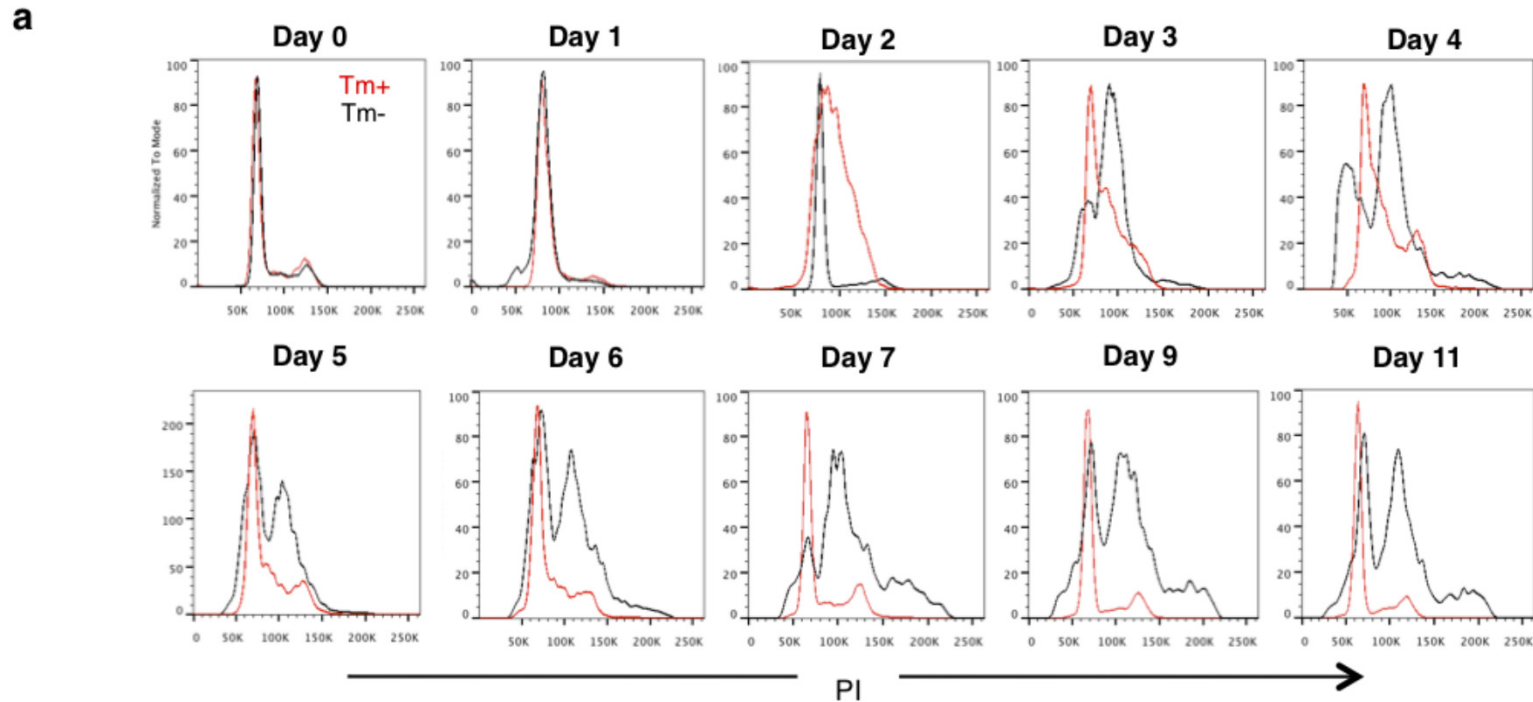
Extended Data Figure 4 | Cell viability of purified Tm⁺CK14⁺ and Tm⁻CK14⁻ cancer cells after GC chemotherapy treatment *in vitro* (raw FACS data for Fig. 1h). Dot plots depict FACS analyses showing cell viability of Tm⁺CK14⁺ (red) and Tm⁻CK14⁻ (black) cancer cells following

11 consecutive days of chemotherapy treatment *in vitro*. The percentage of viable cells defined as annexin V⁻PI⁻ is shown in the bottom left quadrant of each plot. Experiments were performed in biological duplicates.

Zellzyklusanalyse



Zellzyklusanalyse

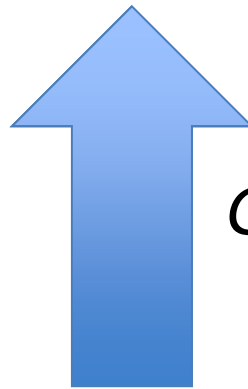


Extended Data Figure 5 | Cell cycle profiles of purified $Tm^+ CK14^+$ and $Tm^- CK14^-$ cancer cells after GC chemotherapy *in vitro* (raw FACS data for Fig. 1i). Histogram plots depict original FACS analyses of cell cycle profiles

from $Tm^+ CK14^+$ (red) and $Tm^- CK14^-$ (black) cancer cells after 11 consecutive days of chemotherapy treatment *in vitro*. Experiment was performed in biological duplicates. PI, propidium iodide.

Im Überblick

- **Tm⁺ CK14⁺ Zellen** stellen die gesuchten CK14⁺ CSCs dar
- **tumorresistenter als Tm⁻CK14⁻ CSCs**

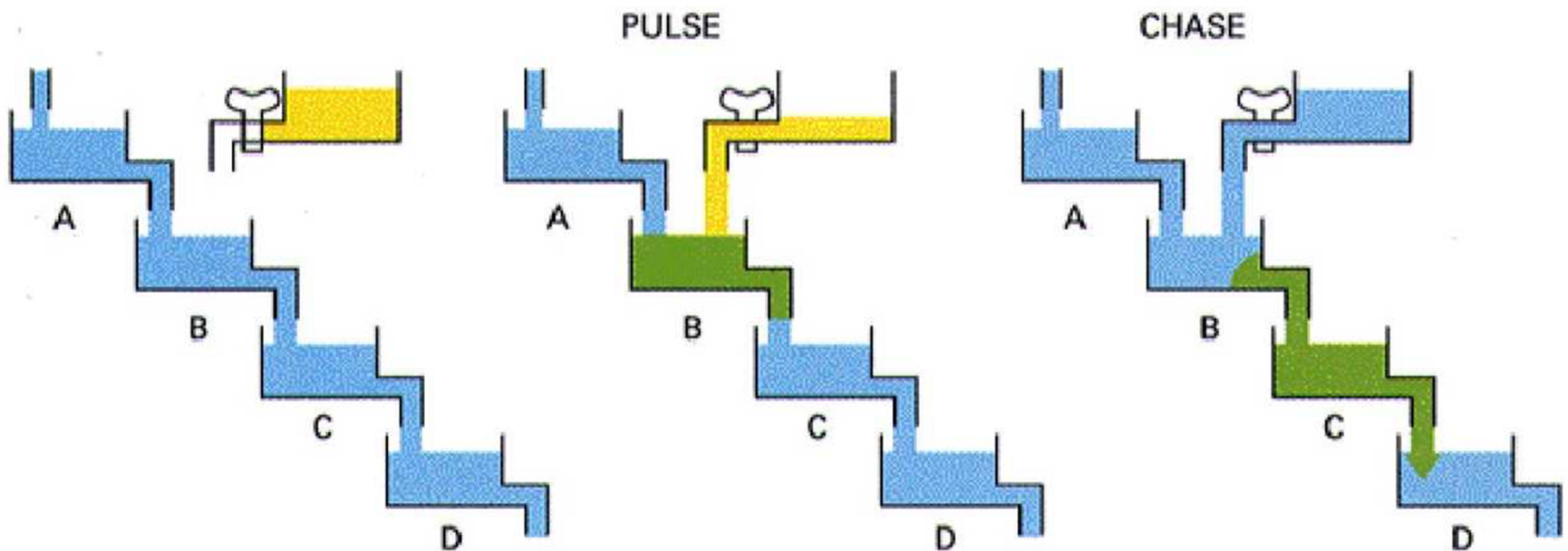


Grund?

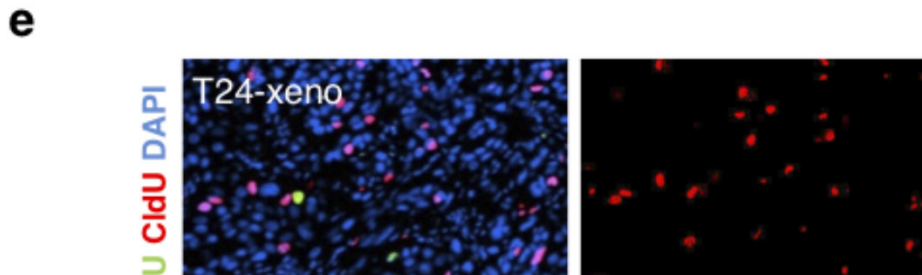
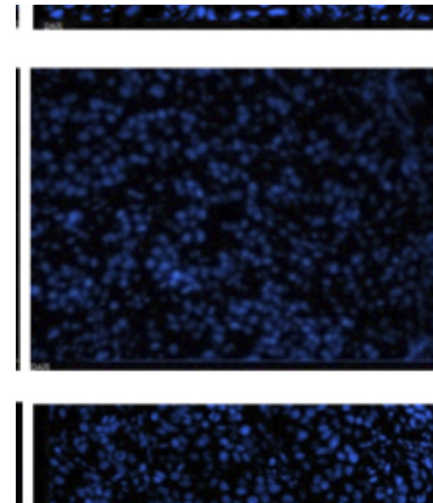
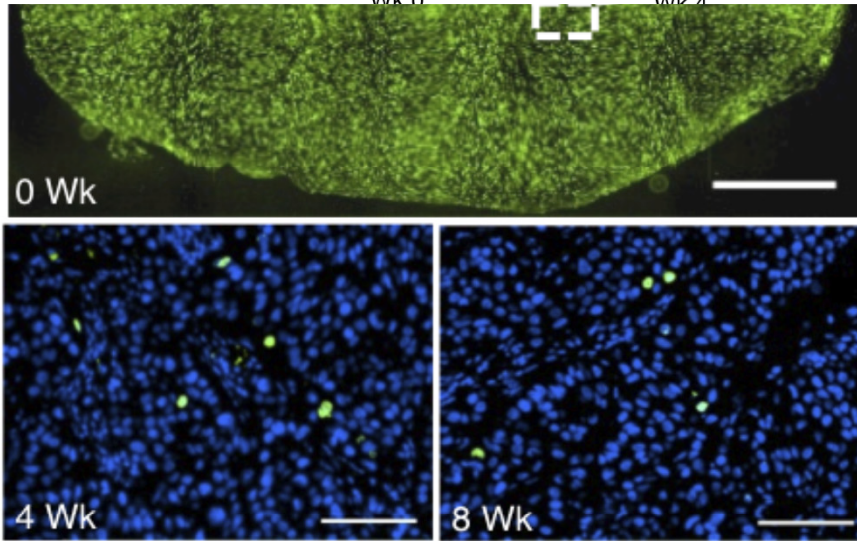
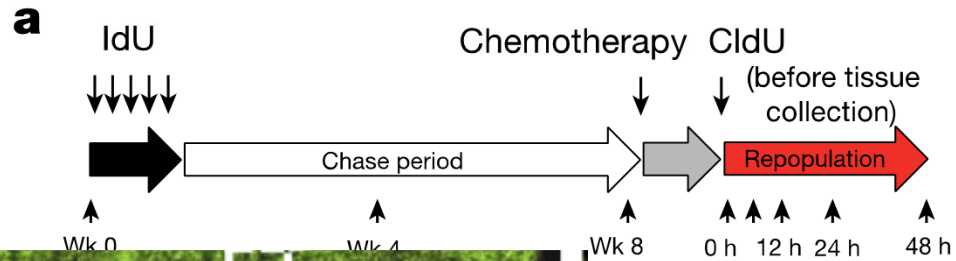
- **Weniger proliferativ**

Untersuchung der Proliferation

„Pulse-chase-methode“

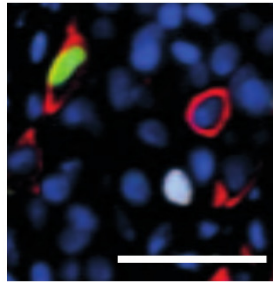


Untersuchung der Proliferation

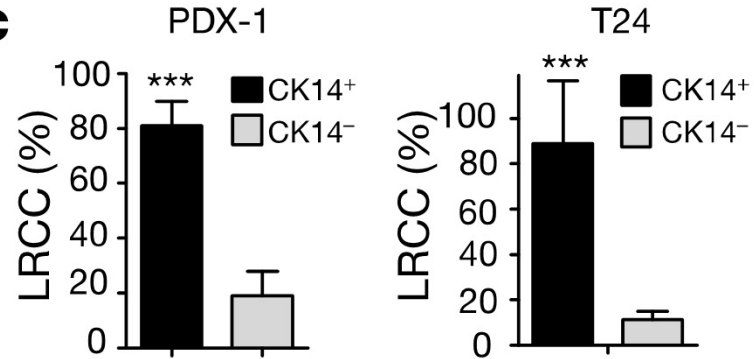


Untersuchung der Proliferation

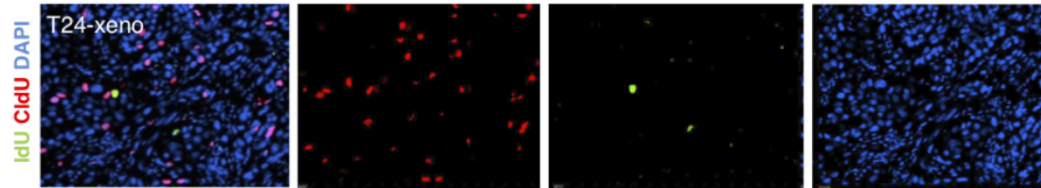
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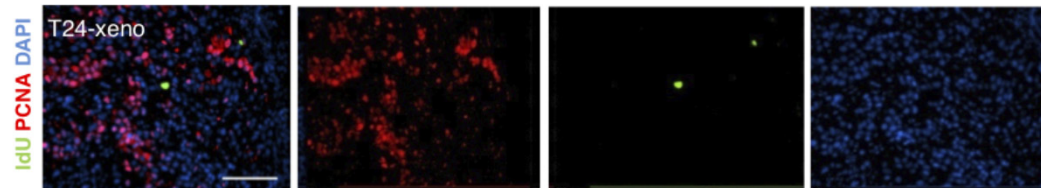
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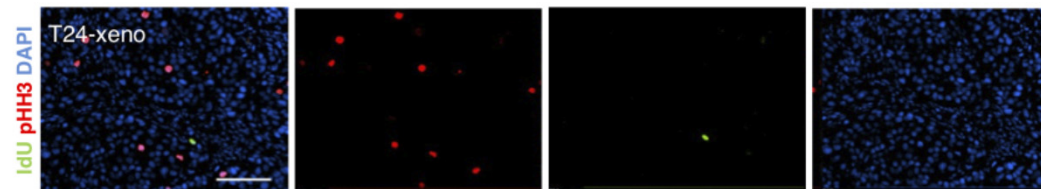
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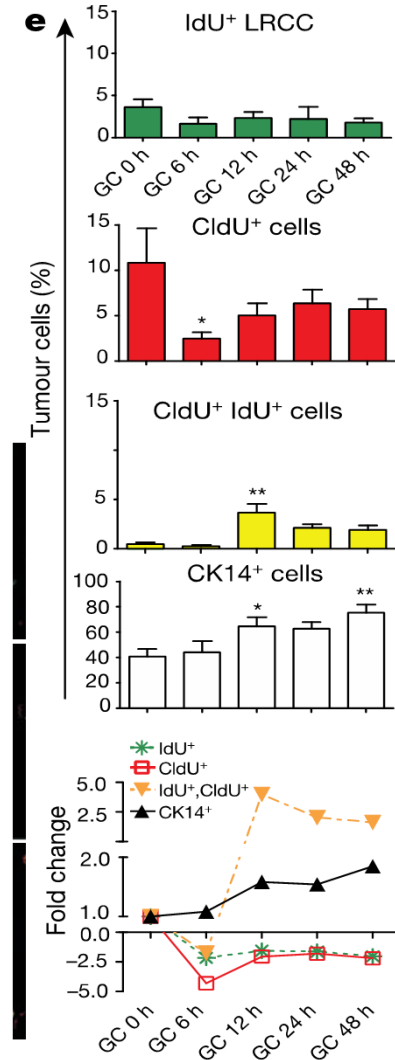
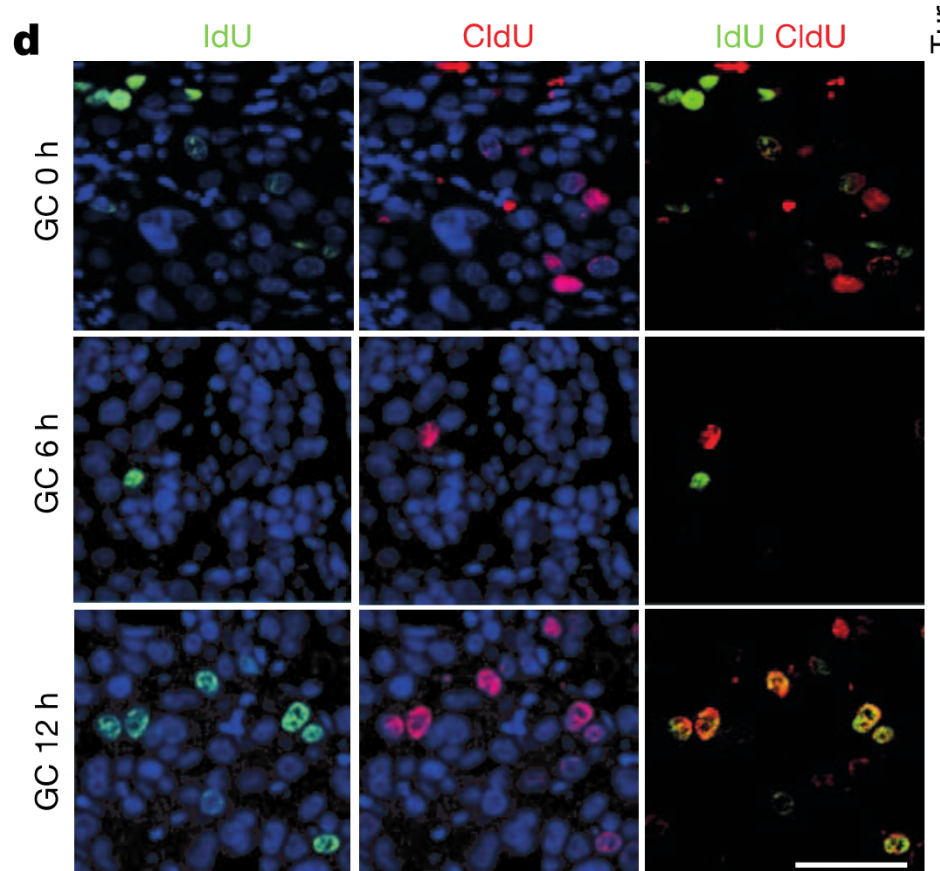
g



Im Überblick

- **Untersuchung der Proliferation** von CK14+ Zellen im Vergleich zu CK14- CSCs
- **IdU+ LRCCs** → gemeinsam mit CD14+ lokalisiert
- **CIDu+ LRCCs** → nicht mit CD14+ gemeinsam lokalisiert

Untersuchung der Proliferation

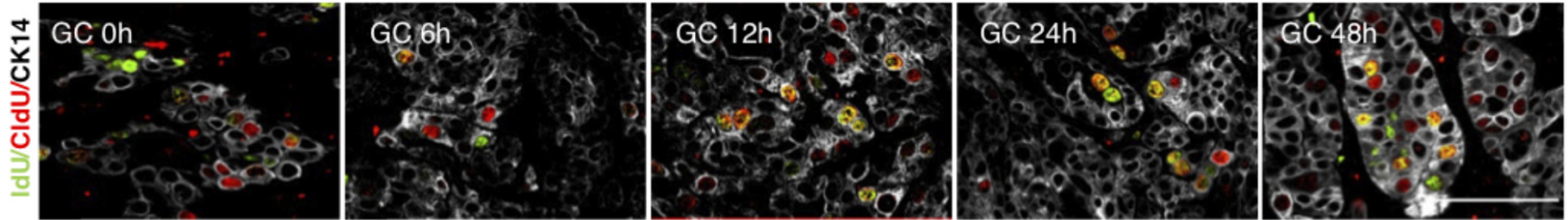




Untersuchung der Proliferation

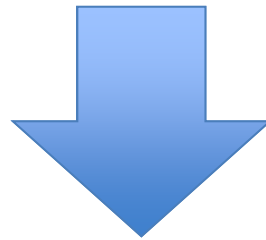


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Im Überblick

LRCCs Proliferation + CK14⁺ Zunahme
nach Chemotherapie (hier nach 12h)



„Tumorwiederbesiedlung / *residual tumors*“

Mechanismus d. Wiederbesiedlung

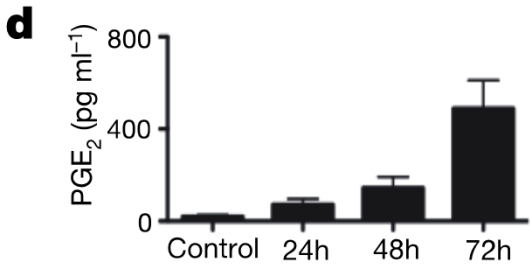
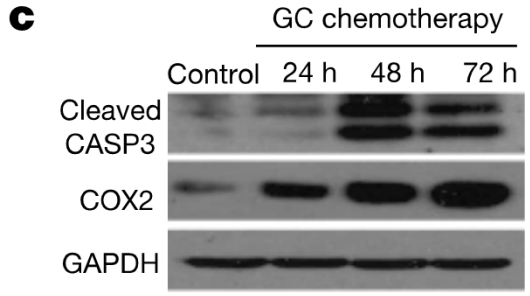
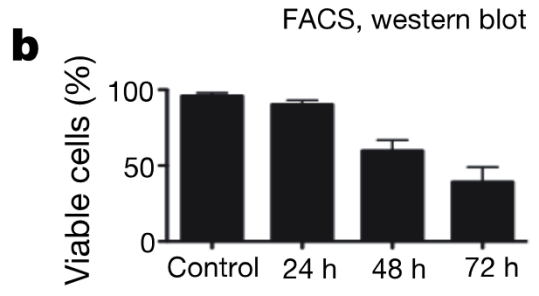
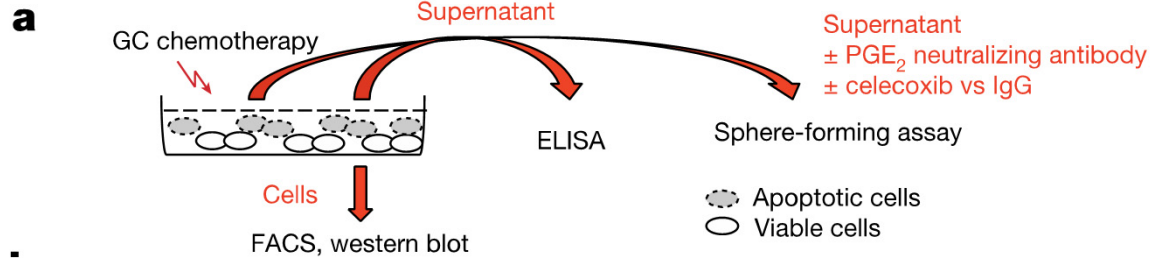
Chemotherapie



Zellen

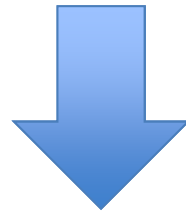


Apoptose → lösliche Faktoren, zB. PGE2



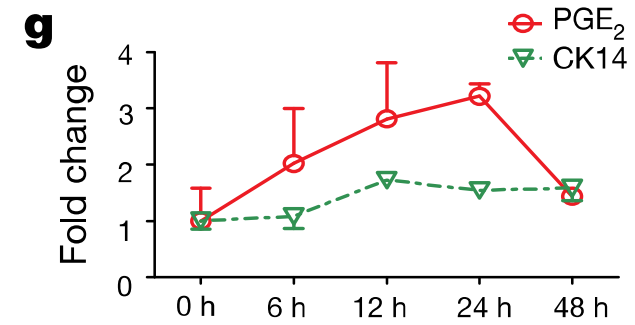
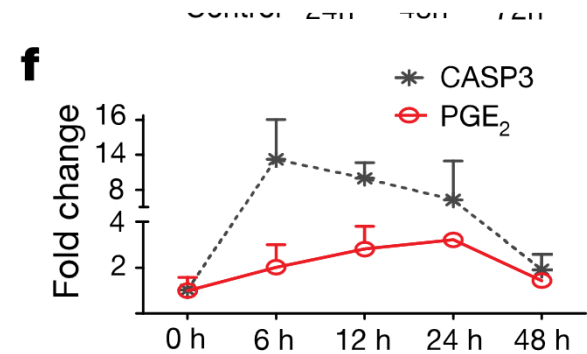
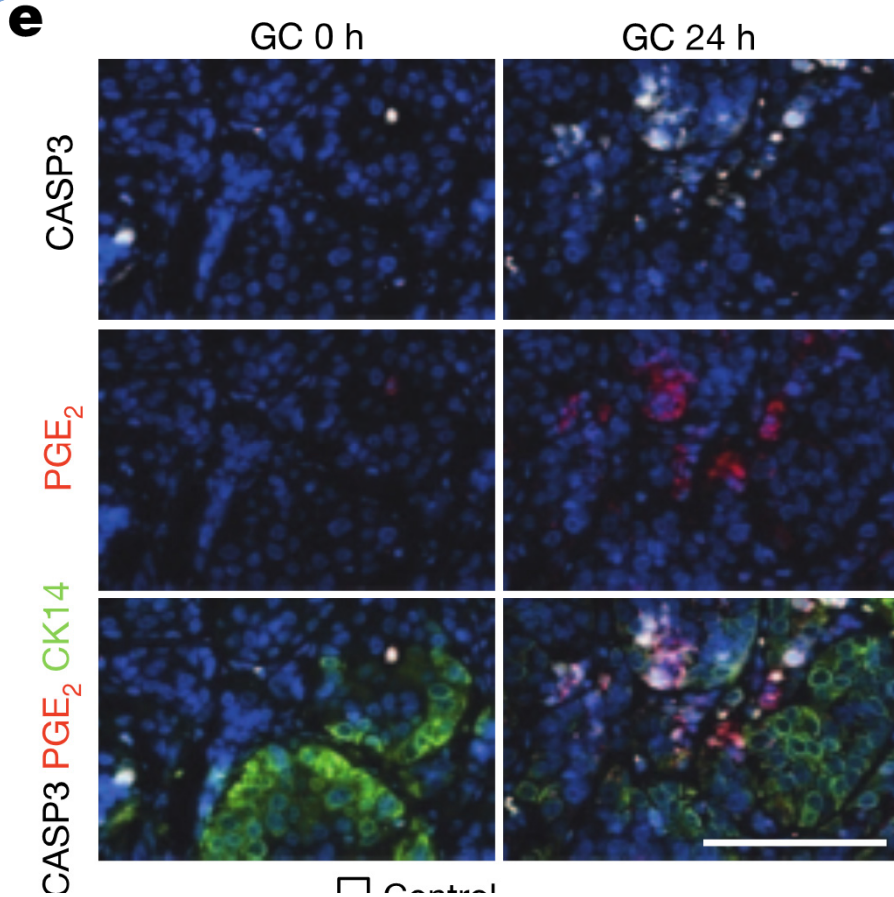
Im Überblick

Beginnende Apoptose



Erhöhte Expression von COX-2 und PGE2

Mechanismus der Wiederbesiedlung



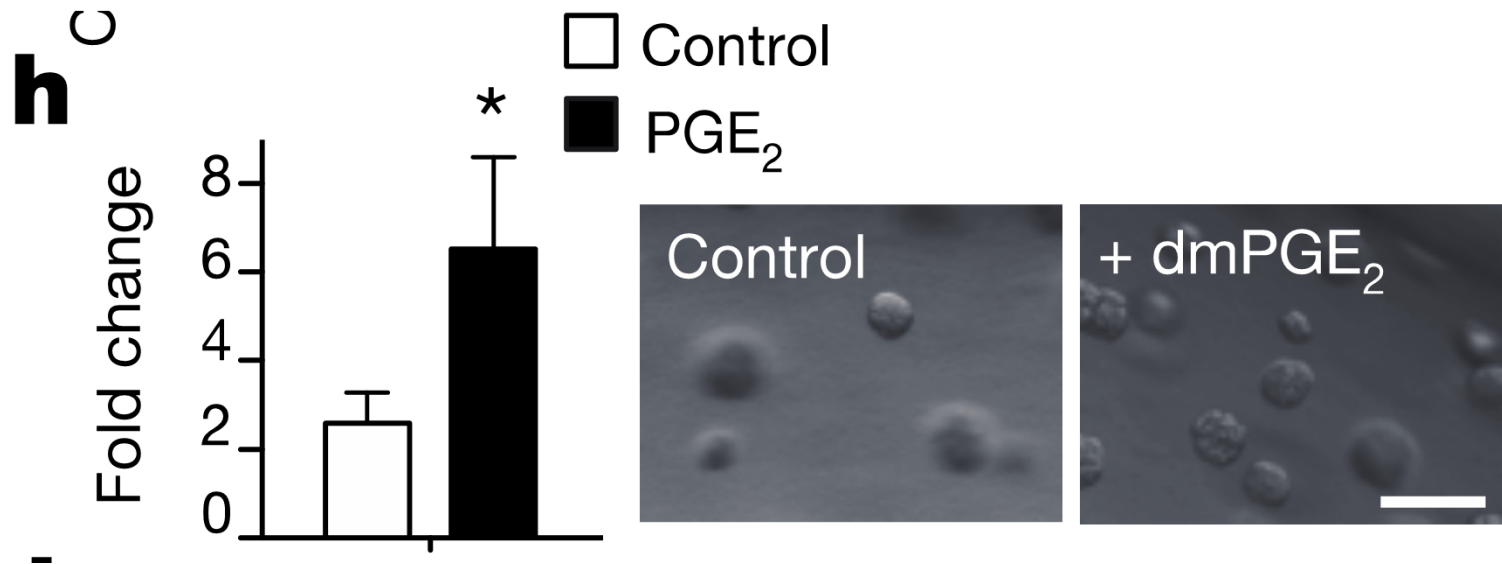
Im Überblick

Chemotherapie-ind. Apoptose → PGE₂
Ausschüttung

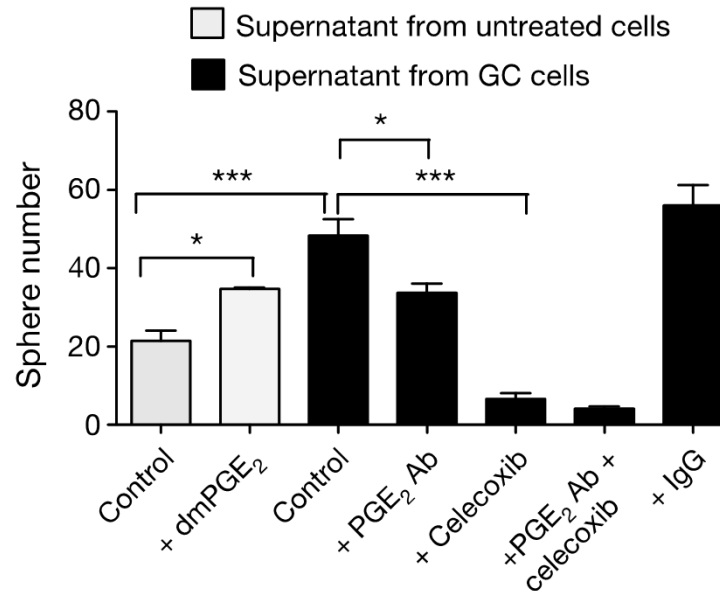
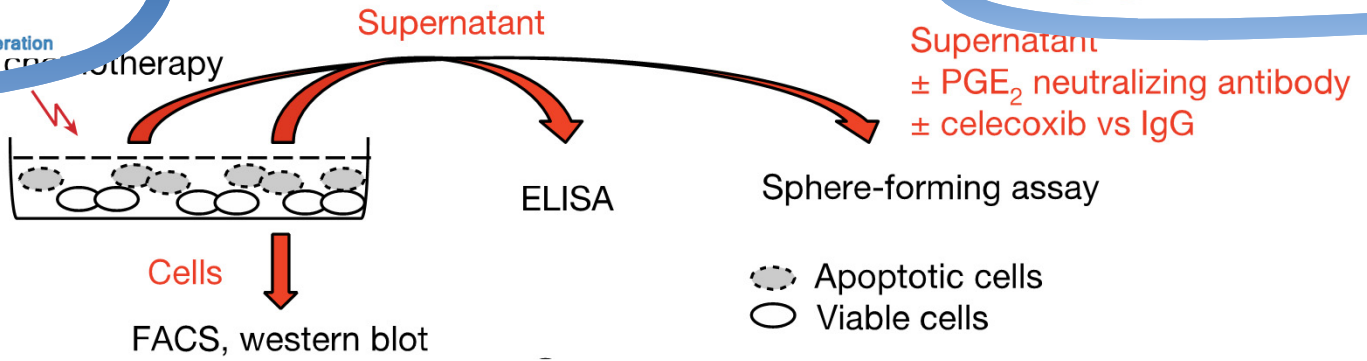
PGE₂-Ausschüttung vor Tumorwiederbesiedlung

Die Rolle von PGE₂

„Sphere-forming“



GC cell therapy

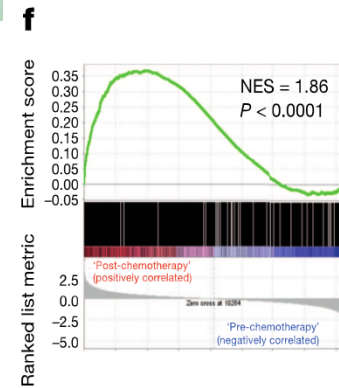
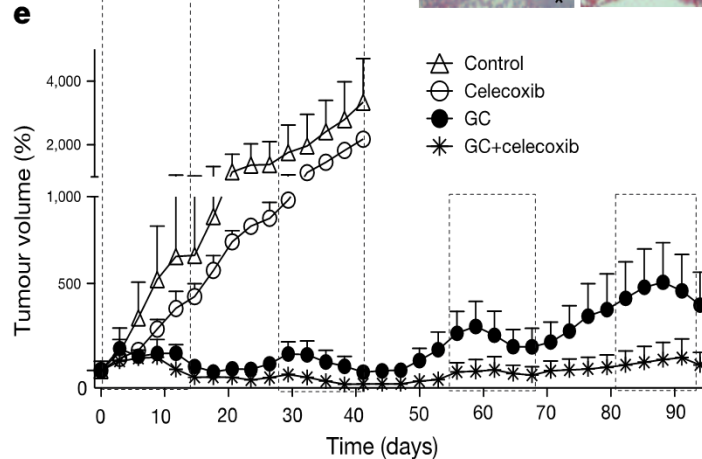
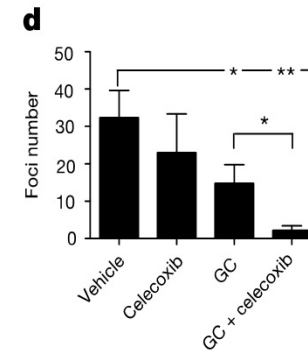
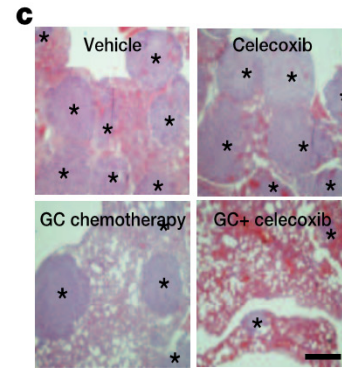
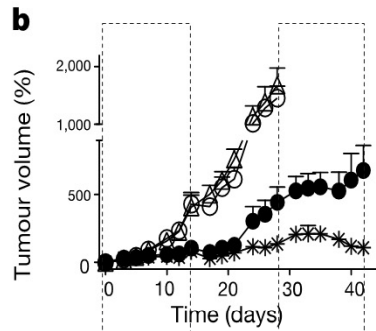
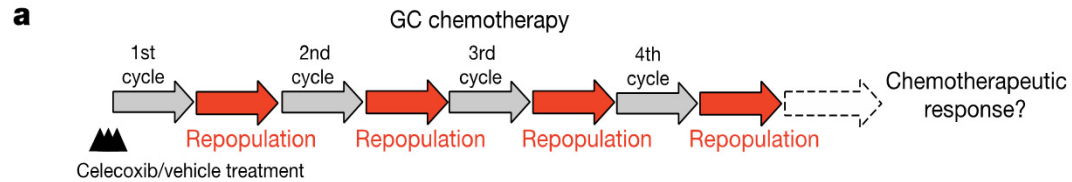


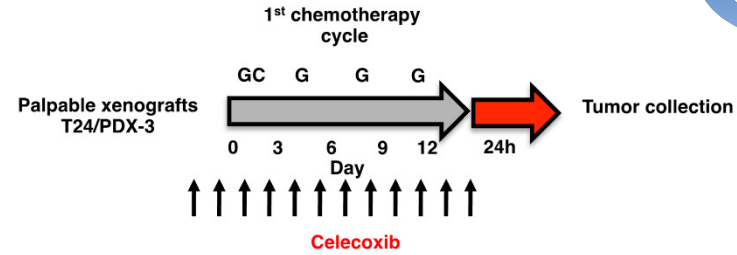
Im Überblick

- In vitro:

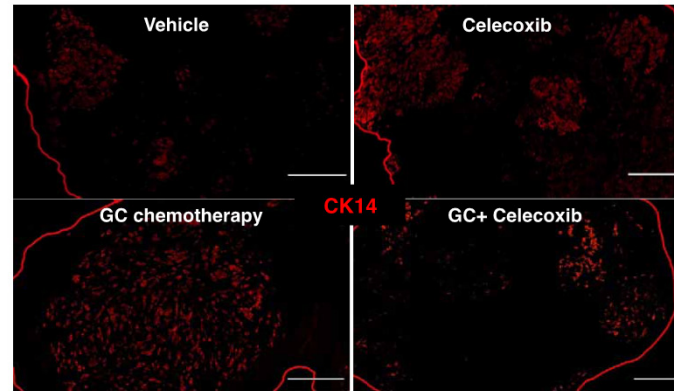
Hemmung von PGE2 senkt
„sphere-forming“-Aktivität

Hemmung von PGE2 in vivo

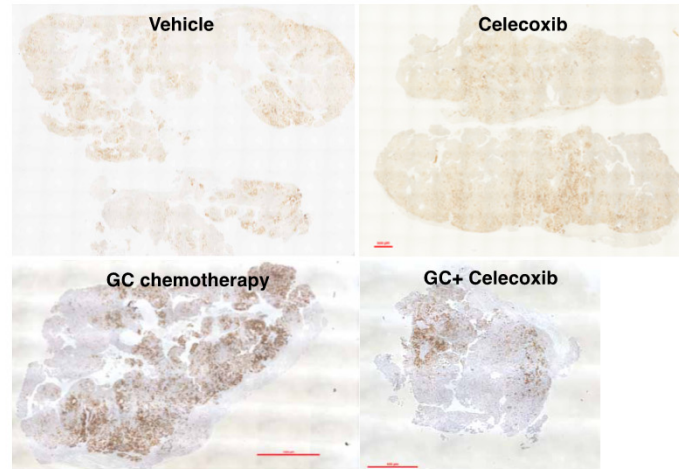




b

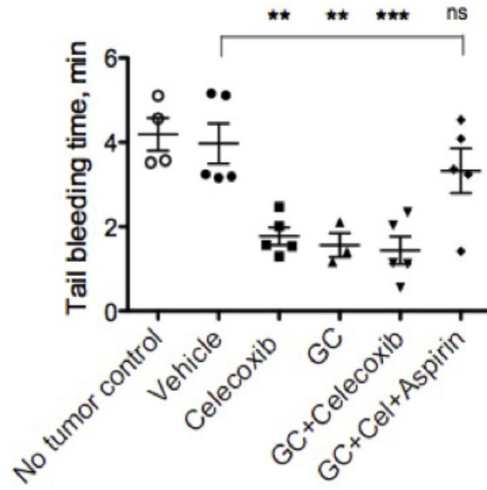


c

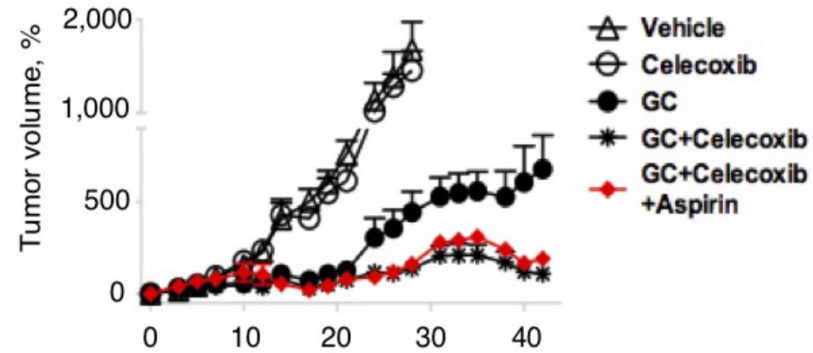


Aspirin & Celecoxib

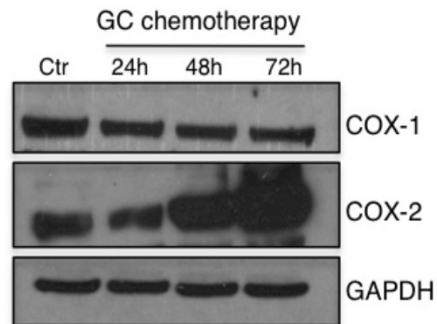
a



b

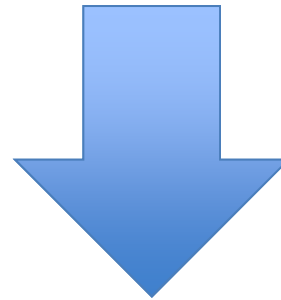


c



Im Überblick

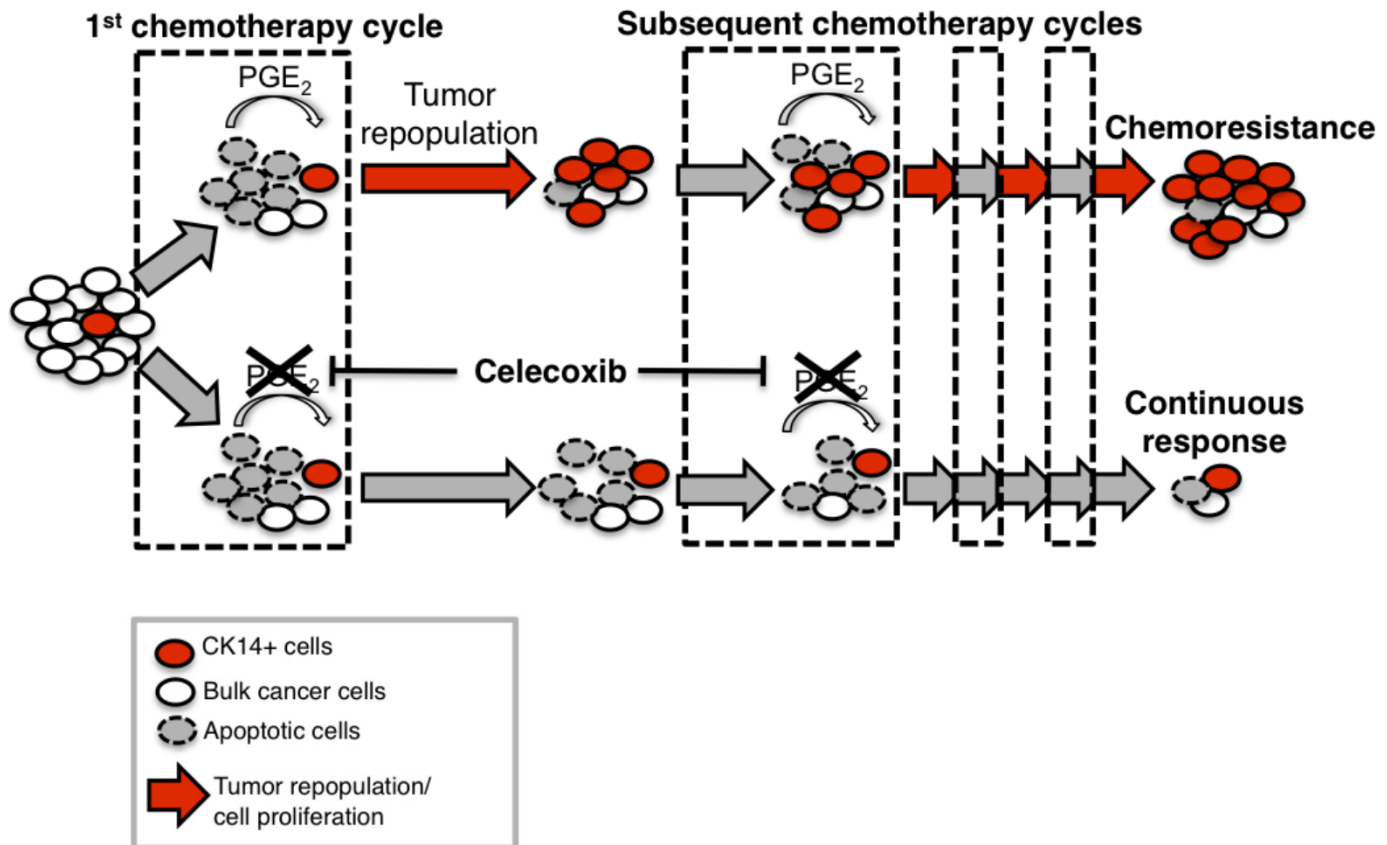
- Chemotherapie in Kombination mit Celecoxib



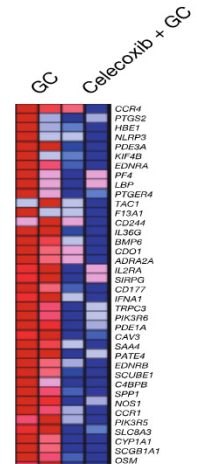
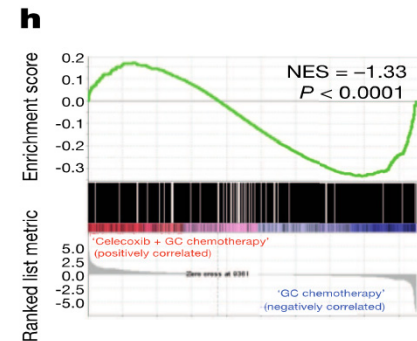
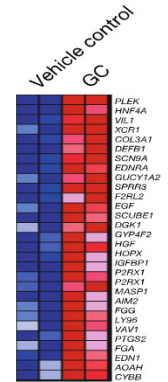
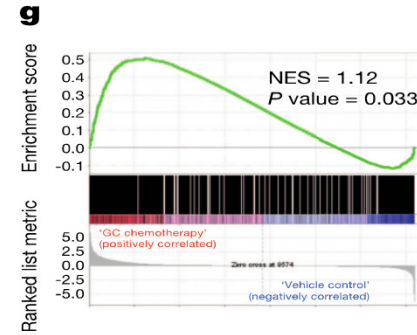
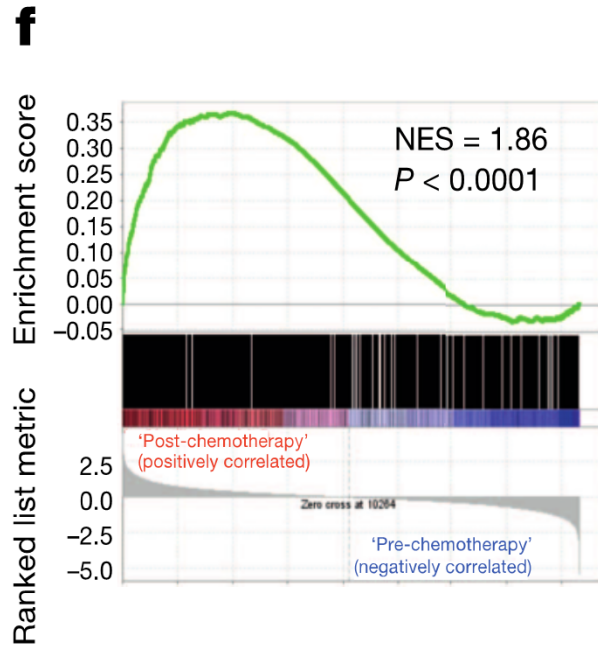
Verbessert Chemotherapieansprechen

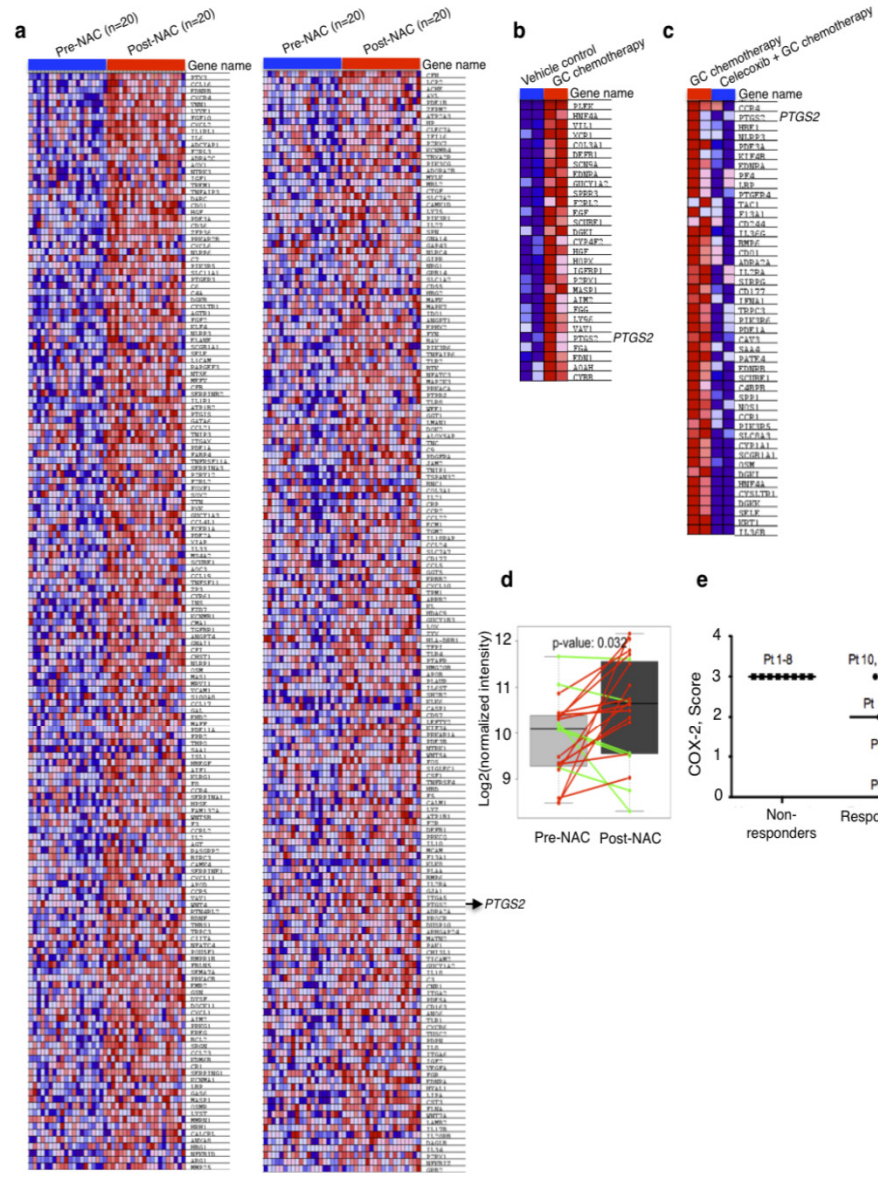
- Dazu Aspirin → keine Nachteile

LETTER RESEARCH



Global gene-expression profile





Zusammenfassung

PGE₂/COX₂-Signalweg → Rolle bei
Chemo-Resistenz

Celecoxib → Chemoresistenz 

Klinik, andere Tumorarten...

Danke für die Aufmerksamkeit