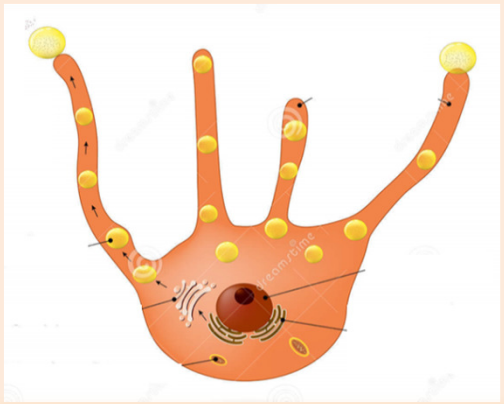
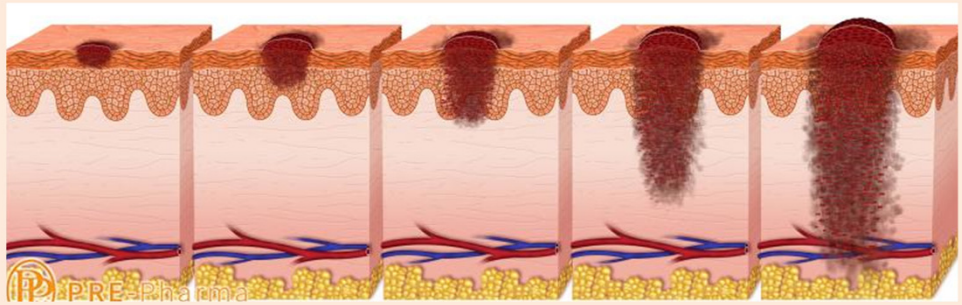
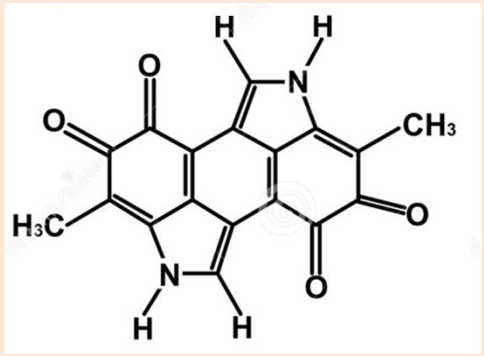




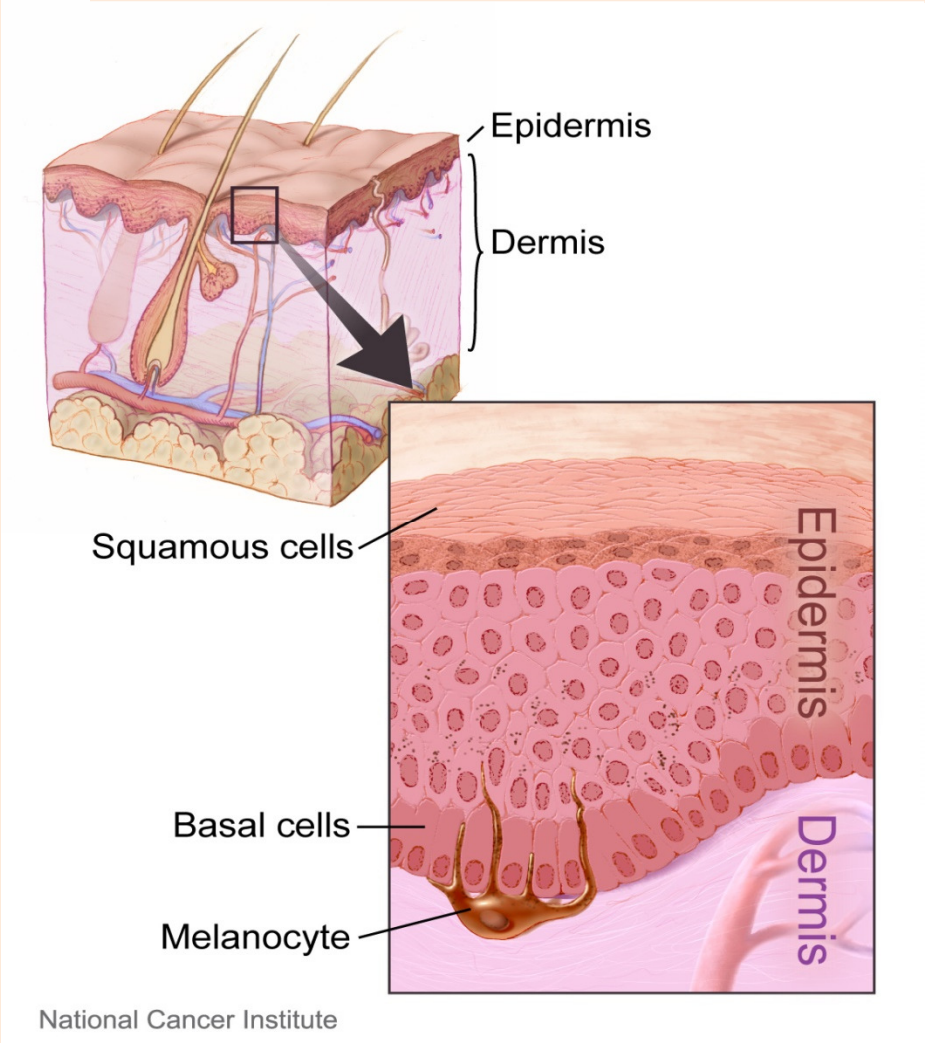
Bioconjugates for treatment of metastatic melanoma - difficulties and challenges



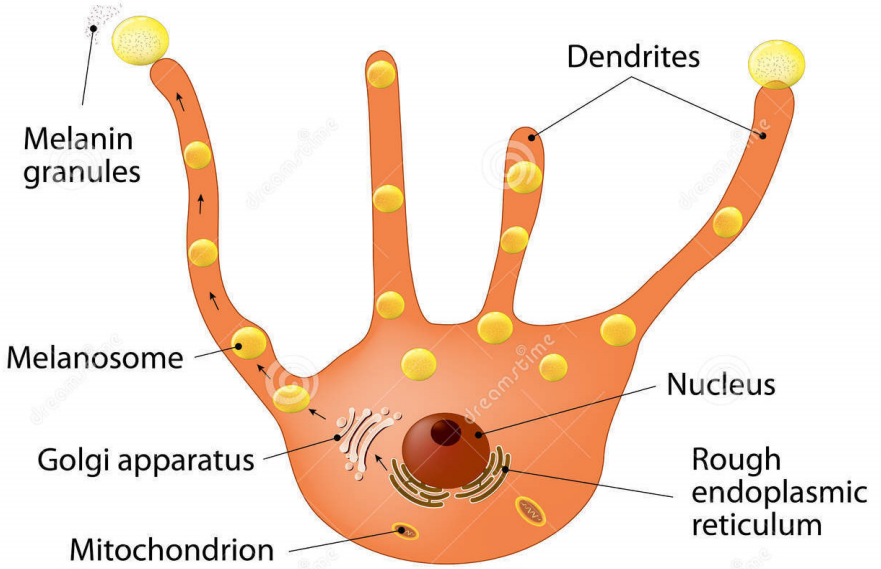
Ildikó Szabó

02.05.2019.

Anatomy of skin, melanocytes



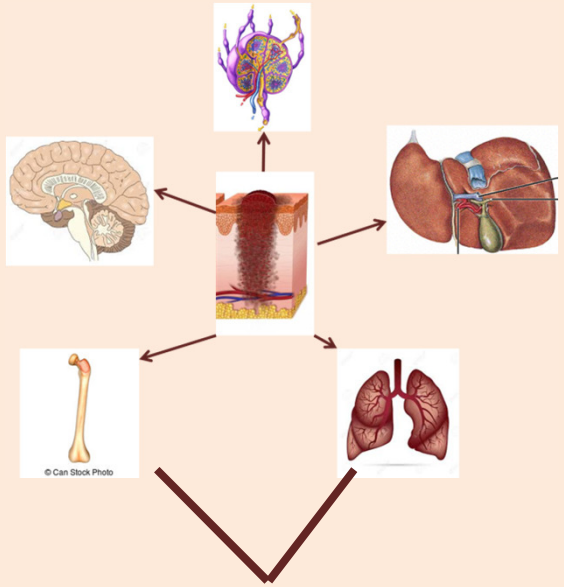
https://commons.wikimedia.org/wiki/File:Layers_of_the_skin.jpg



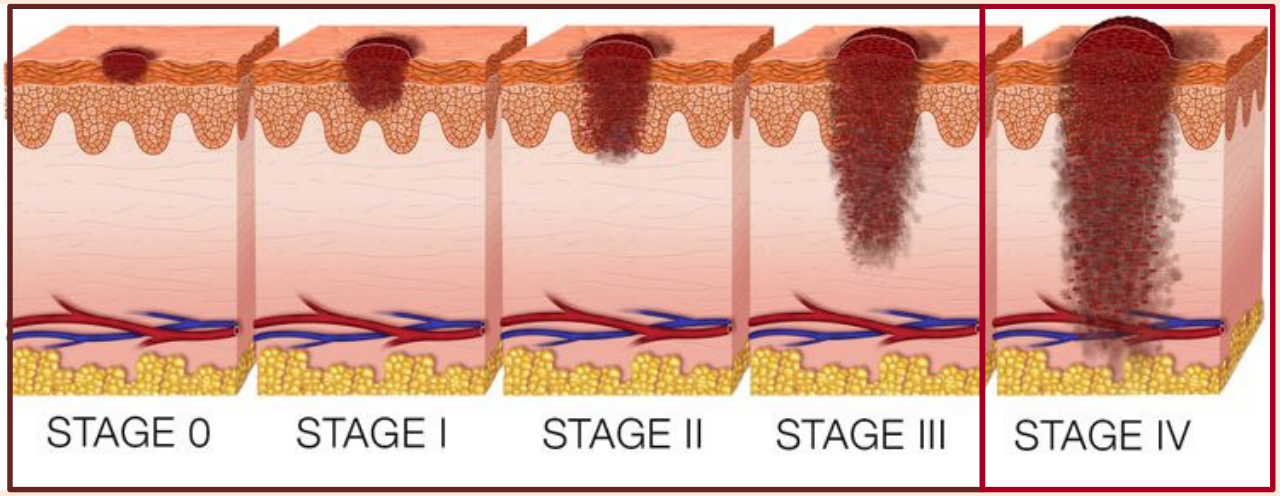
https://www.dreamstime.com/stock-illustration-melanocyte-melanin-melanogenesis-melanin-producing-cells-melanin-pigment-responsible-skin-color-image54608996

Melanoma

- develops in melanocytes
- melanocyte form moles by aggregation
- moles begin to grow and divide in an uncontrolled way
- most serious form of cancer, it can grows very quickly if left untreated
- spread to lower part of skin enter bloodstream and lymphatic system forming metastasis



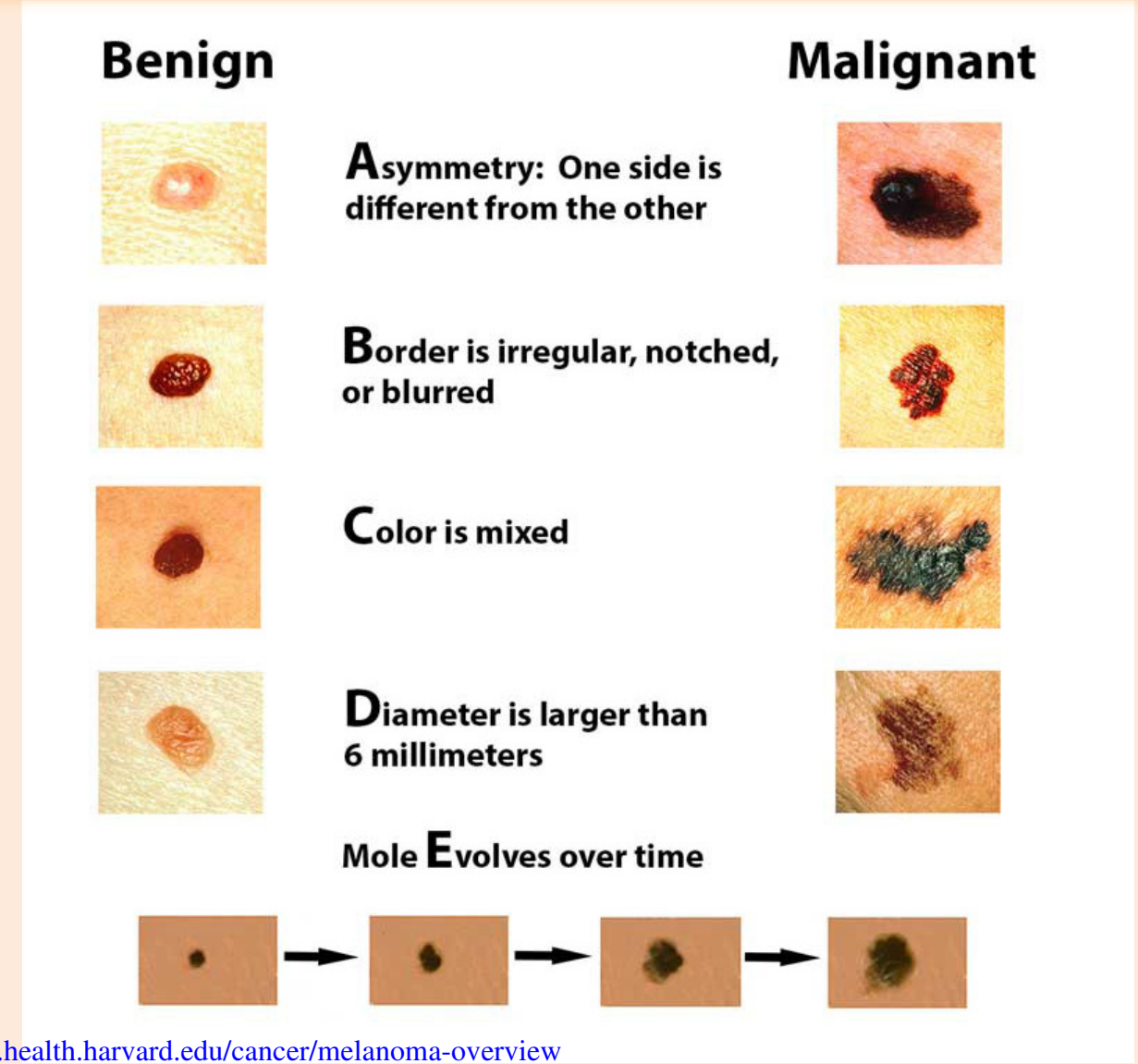
Good prognosis



Treatment!

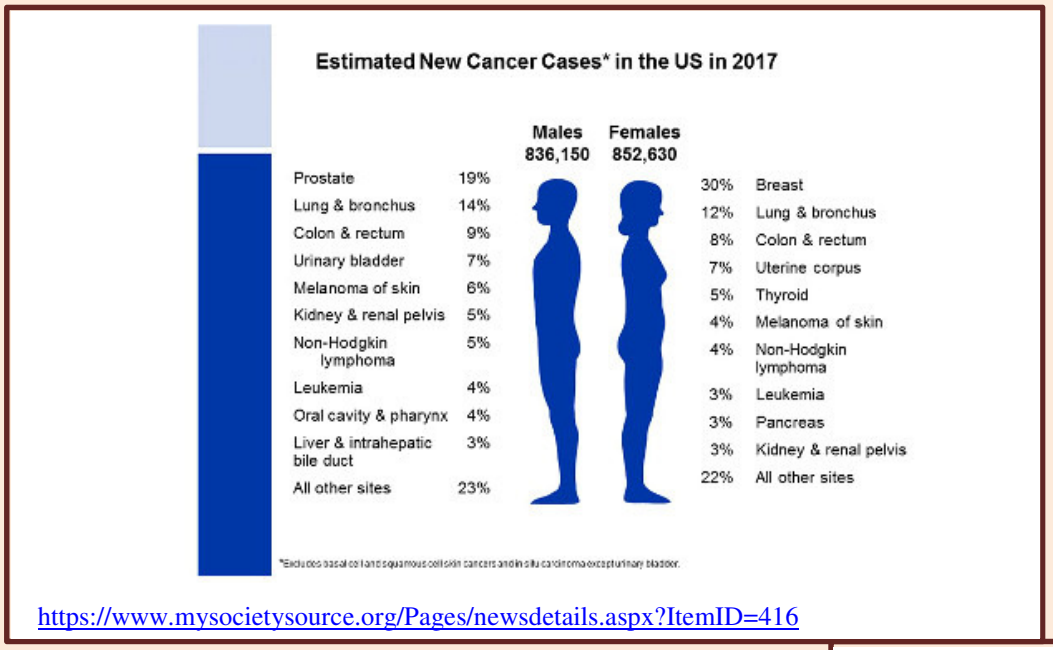
https://www.wellnesscommunitydc.org/skin-cancer-stages/

The ABCDE's of melanoma



Forrás: https://www.health.harvard.edu/cancer/melanoma-overview

Cancer statistics



https://www.mysocietysource.org/Pages/newsdetails.aspx?ItemID=416

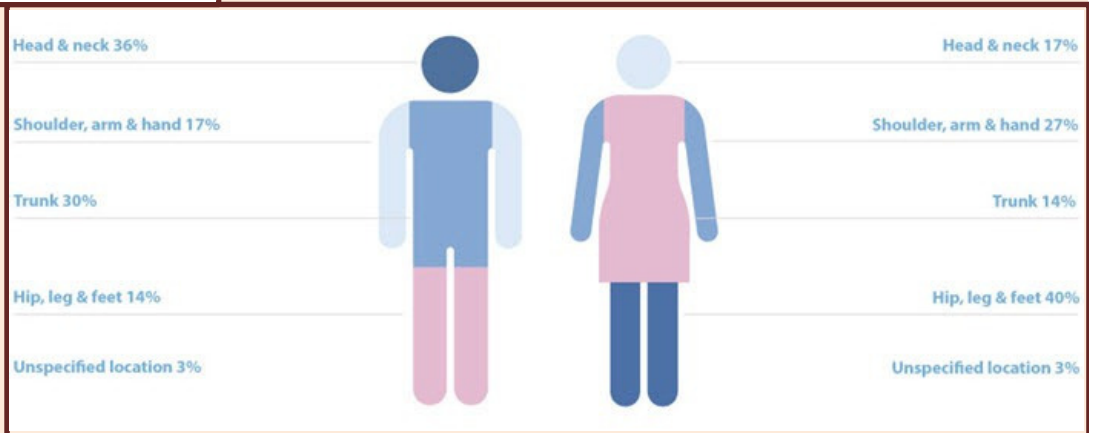
- Cancer of the skin is most common of all cancers
- Melanoma account only 1% of skin cancers
- Rates of melanoma have been rising
  - Ageing population

Importance of the sun-bathing and usage of sun-beds

↓

Overexposure UVB radiation:

- Sunburn
- Skin cancer due to direct DNA damaging effect



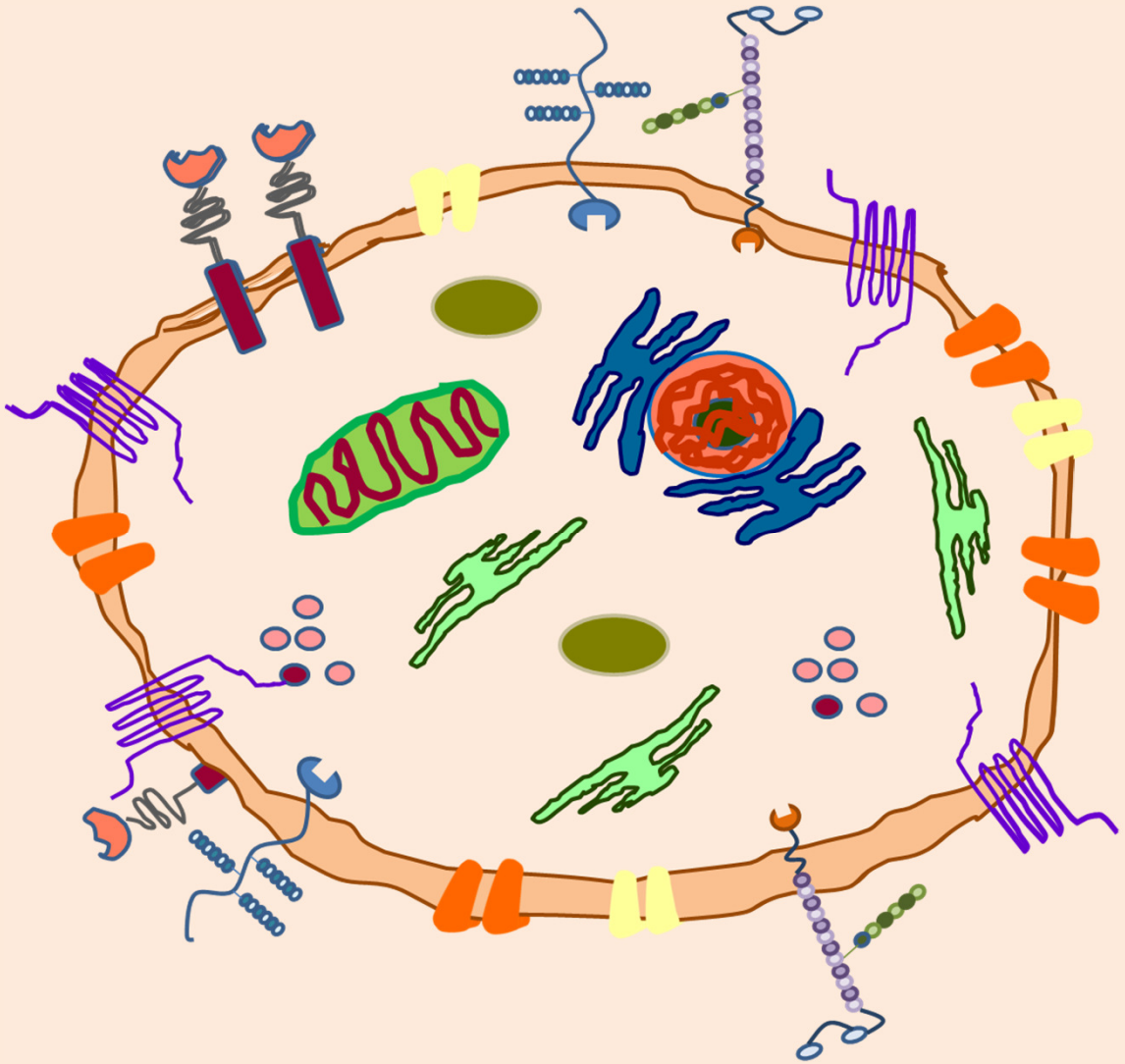
https://careinthesun.org/skin-cancer/skin-cancer-statistics/

Treatment of Melanoma
facts, posibilities, difficulties

- For determination of melanoma biopsy is applied
- Based on the laboratory examination:
  - Cancerous or not
  - How deep has it grown
- Early detected melanoma can be effectively treated
  - surgery, biopsy
  - Sentinel lymph node is cancer cell free
- In case of metastasis:
  - One or more lymph node contain cancer cells→ quickly get to other organs
  - Chemotherapy, immunotherapy
  - The overall success in metastatic melanoma is quite limited

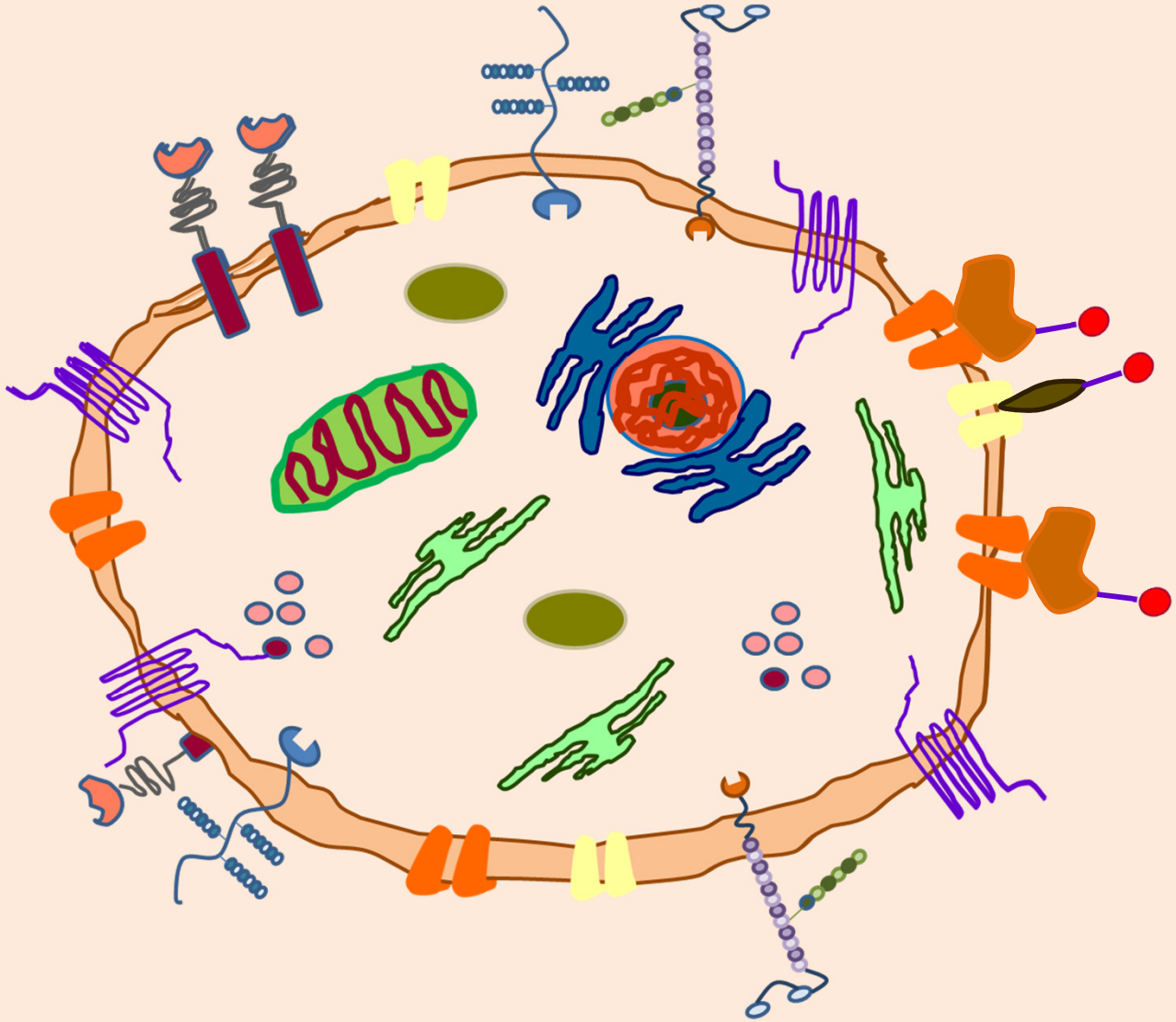
Possibilities of melanoma treatment

targeted tumor therapy



Possibilities of melanoma treatment

cancer cell specific cell surface molecules



Phage display technology

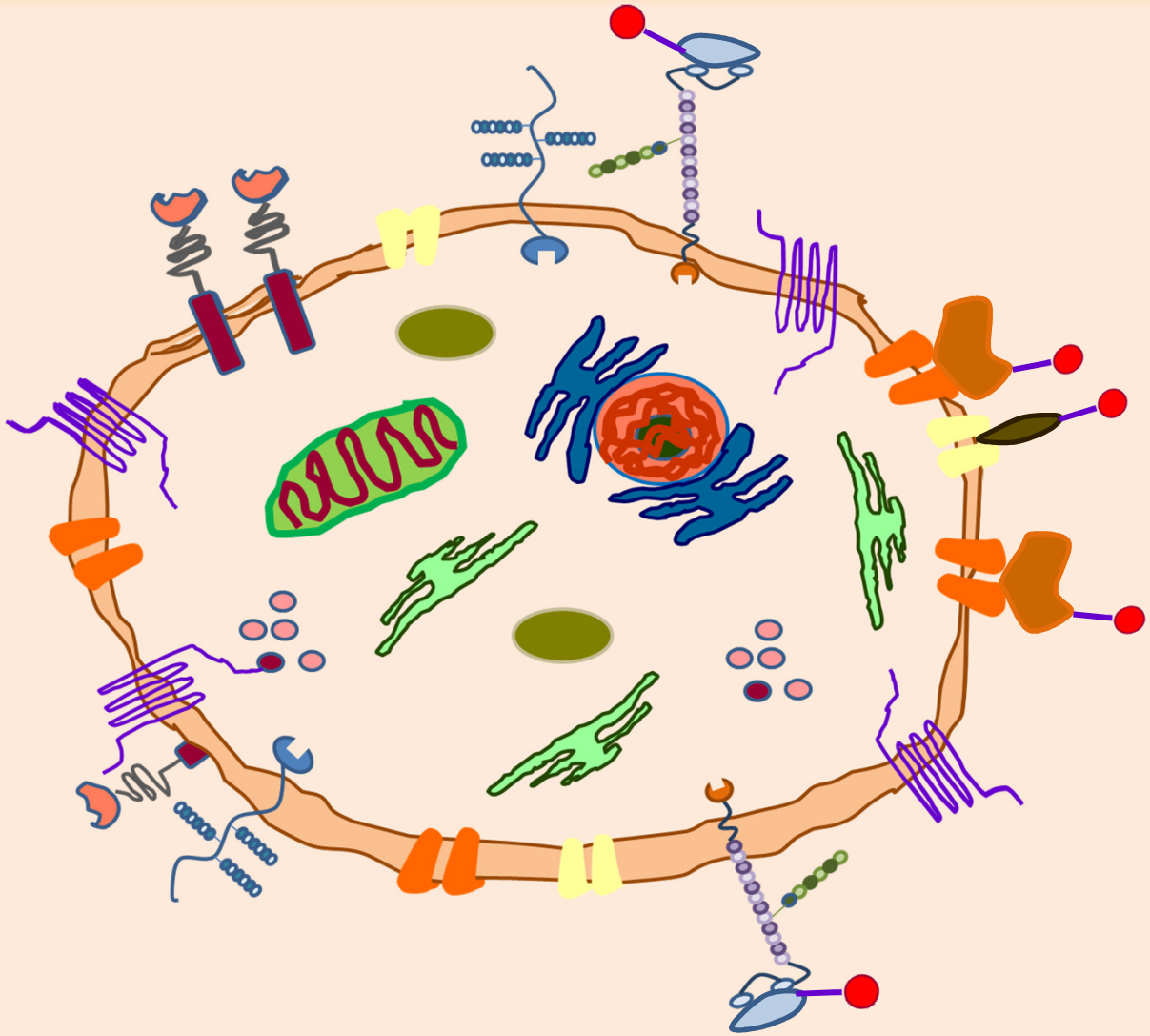
- Principle screening for specific peptides that bind to target from a library of phage particles
- Peptides binding to individual targets can be identified by affinity selection (biopanning)
- Phage-displayed peptide library can be used:
    - B-cell and T-cell epitop mapping
    - selection of bioactive peptides bound to receptors or proteins
    - selection of disease specific antigen mimics
    - selection of peptides bound to non-protein targets
    - selection of cell specific peptides
    - selection of organ-specific peptides
    - development of peptide mediated drug delivery systems
- Targeting peptides have potential use in basic research and translational medicine.

Smith, G.P. *et al* Science (1985) **228**, 1315-1317

Wu, C-H. *et al*. J. Med. Sci (2016) **23**, 8-22.

Possibilities of melanoma treatment

targeting proteoglycans



CSPG4/NG2 proteoglican

- Melanoma-associated chondroitin sulphate proteoglycan (MCSP)
- Transmembrane proteoglican („single-pass, type-I transmembrane proteoglycan)
- Highly immunogenic tumor antigen of melanoma tumor cells
- It has been subsequently detected in various species (e.g. Human, mouse, rat)

Structure of CSPG4/NG2

N-terminal domain: two laminin-like globular (LG) repeats; mediate ligand binding, cell–matrix and cell–cell interactions, interaction with integrins and receptor tyrosine kinase

Central subdomain (D2): 15 tandem repeats of a new module called CSPG (cell–matrix interaction, bind to collagen V és VI, FGF and PDGF

Intracellular domain (D3): bind to integrins, galectin and numerous proteas cleavage sites

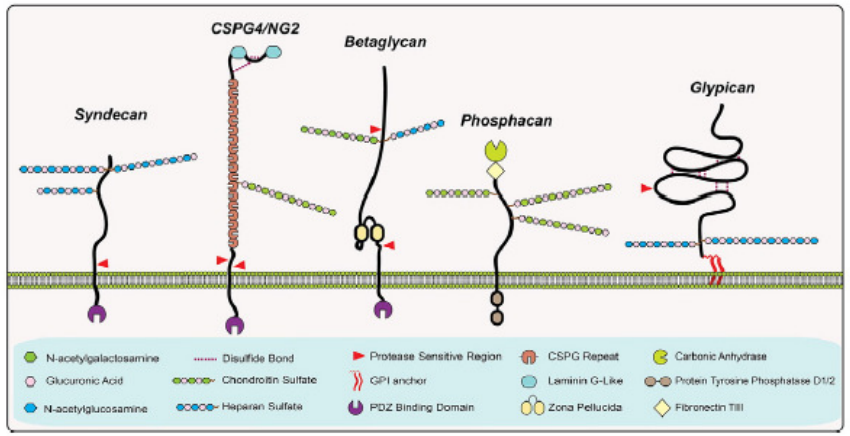


Fig. 2. Schematic representation of the cell surface proteoglycans, which comprise transmembrane type I (the N-terminus is outside of the plasma membrane) proteoglycans (four syndecans, CSPG4/NG2, betaglycan and phosphacan) and six GPI-anchored proteoglycans, glypicans 1–6. The type of GAG chain and the major protease sensitive sites are indicated. The key for the various modules is provided in the bottom panel.

Iozzo, R.V.; Schaefer, L. Matrix Biol (2015) **42**, 11-55.

Specific NG2-binding peptides

have been used phage display to isolate peptides that bind to the NG2 proteoglycan and home to NG2-expressing tumor neovasculature

LTLRWVGLMS „Peptide 1”

TAASGVRSMH „Peptide 2”

- have high affinity and specificity to NG2 proteoglycan
- Binding to BSA is minimal compared to the proteoglycan
- Peptides bind to similar sites on NG2

Guan, Y-Y *et al*. Biomaterials (2014) **35**, 3060-3070
Burg, MA *et al*. Cancer Research (1999) **59**, 2869-2874.

Specific NG2-binding peptide conjugates I.

„Peptide 1”

LTLRWVGLMS

Dau=Aoa-LTLRWVGLMS

Dau=Aoa-GFLG-LTLRWVGLMS

Dau=Aoa-LTLRWVGLNleS

Dau=Aoa-GFLG-LTLRWVGLNleS

„Peptide 2”

TAASGVRSMH

Dau=Aoa-TAASGVRSMH

Dau=Aoa-GFLG-TAASGVRSMH

Dau=Aoa-TAASGVRSNleH

Dau=Aoa-GFLG-TAASGVRSNleH

Chemical characterization of drug containing NG2 conjugates

	Conjugates	t_R (min)[a]	M_{calc}	M_{meas}[b]
SzI-1	Dau=Aoa-TAASGVRSMH-NH_2	10.6	1596.6	1596.5
SzI-10	Dau=Aoa-GFLG-TAASGVRSMH-NH_2	13.5	1970.5	1970.9
SzI-2	Dau=Aoa-LTLRWVGLMS-NH_2	14.4	1756.1	1756.2
SzI-3	Dau=Aoa-TAASGVRSNleH-NH_2	13.9	1578.1	1578.8
SzI-4	Dau=Aoa-GFLG-TAASGVRSNleH-NH_2	13.7	1952.5	1953.1
SzI-5	Dau=Aoa-LTLRWVGLNleS-NH_2	15.1	1737.5	1737.2
SzI-6	Dau=Aoa-GFLG-LTLRWVGLNleS-NH_2	18.1	2112.3	2112.4
	Dau=Aoa-GFLG-LTLRWVGLMS-NH_2	-	2130.8	-

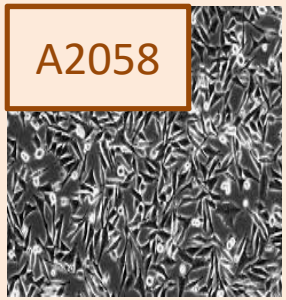
[a] Analitical RP-HPLC, Agilent Eclipse XDB C8, 5 µm, 80Å, 4.6 x 150 mm, HPLC coloumn, gradient: 5% B, 2 min; 5-100% B, 20 min.

[b] Bruker Daltonics Esquire 3000plus (Bremen, Germany) ion trap mass spectrometer. Spectra were acquired in the 50–2000 m/z range

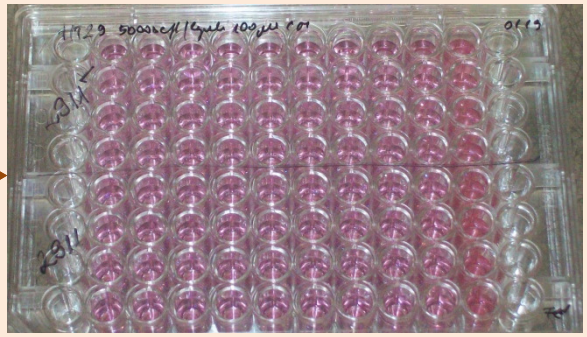
Determination of *in vitro* cytostatic effect of drug containing NG2 conjugates

MTT-assay

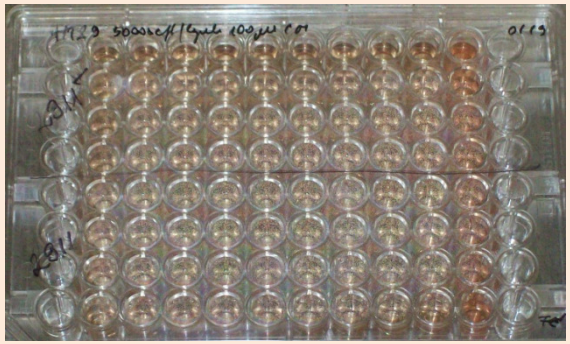
A2058



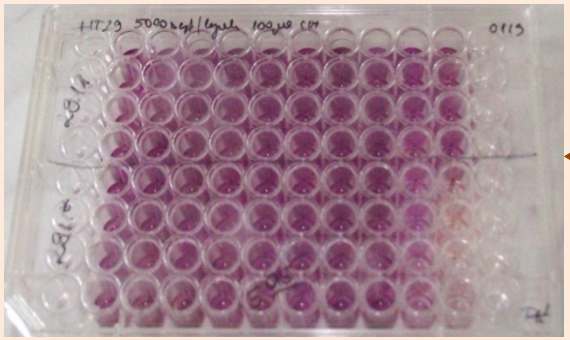
(24h, 37°C)



Treatment
(24h, 37°C, CM,concentration:
0.16/0.8 – 100 µM;
Washing, culturing in FCSM, 48h, , 37°C)



MTT-assay (3.5h, 37°C)



Determination of IC_{50} values

Results

In vitro cytostatic effect of drug containing NG2 conjugates

		IC_{50} (µM)
		A2058
SzI-1	Dau=Aoa-TAASGVRSMH-NH_2	62,8±22,1*
SzI -2	Dau=Aoa-LTLRWVGLMS-NH_2	4,3±1,9
SzI -3	Dau=Aoa- TAASGVRSNleH-NH_2	22,1 és >100*
SzI -4	Dau=Aoa- GFLG-TAASGVRSNleH-NH_2	2,3±1,3
SzI -5	Dau=Aoa-LTLRWVGLNleS-NH_2	17,5±3,3
SzI -6	Dau=Aoa-GFLG-LTLRWVGLNleS-NH_2	26,5±17,6*
SzI -10	Dau=Aoa-GFLG-TAASGVRSMH-NH_2	5,2±2,4
Dau	Dau·HCl	<0.16

Conclusion I.

Structure-activity relationship

„Peptide 1”

LTLRWVGLMS —Met→Nle↓→ **LTLRWVGLNleS**

Szl-2 (4.3±1.9µM) —+GFLG→ Ø

Szl-5 (17.5±3.3µM) —+GFLG→ Szl-6 (26.5±17.6µM) Ø

„Peptide 2”

TAASGVRSMH —Met→Nle Ø→ **TAASGVRSNleH**

Szl-1 (62.8±22.1µM) —+GFLG→ Szl-10 (5.2±2.4µM) ↑

Szl-3 (22.1 és >100µM) —+GFLG→ Szl-4 (2.3±1.3µM) ↑

Specific NG2-binding peptide conjugates II.

„Peptide 1”

LTLRWVGLMS

Dau=Aoa-GFLG-LRWVGLMS

Dau=Aoa-GFLG-WVGLMS

Dau=Aoa-VGLMWSLTRL-NH_2
(scr)

„Peptide 2”

GFLG-TAASGVRSNleH

Dau=Aoa-GFLG-ASGVRSNleH

Dau=Aoa-GFLG-GVRSNleH

Dau=Aoa-GFLG-ARASNleHSTGV-NH_2
(scr)

Chemical characterization of truncated and scrambled NG2 targeted peptide conjugates

Code	Conjugates	t_R (min)[a]	M_{calc}	M_{meas}[b]
SzI-11	Dau=Aoa-VGLMWSLTRL-NH_2 (scr)	17.1	1756.3	1756.5
SzI-12	Dau=Aoa-GFLG-LRWVGLMS-NH_2	16.1	1915.6	1915.4
SzI-13	Dau=Aoa-GFLG-WVGLMS-NH_2	17.4	1645.9	1645.8
SzI-14	Dau=Aoa-GFLG-ARASNleHSTGV-NH_2 (scr)	13.7	1952.5	1952.2
SzI-15	Dau=Aoa-GFLG-ASGVRSNleH-NH_2	13.9	1780.4	1780.5
SzI-16	Dau=Aoa-GFLG-GVRSNleH-NH_2	14.1	1622.2	1622.3

[a] Analitical RP-HPLC, Agilent Eclipse XDB C8, 5 µm, 80Å, 4.6 x 150 mm, HPLC coloumn, gradient: 5% B, 2 min; 5-100% B, 20 min.

[b] Bruker Daltonics Esquire 3000plus (Bremen, Germany) ion trap mass spectrometer. Spectra were acquired in the 50–2000 m/z range

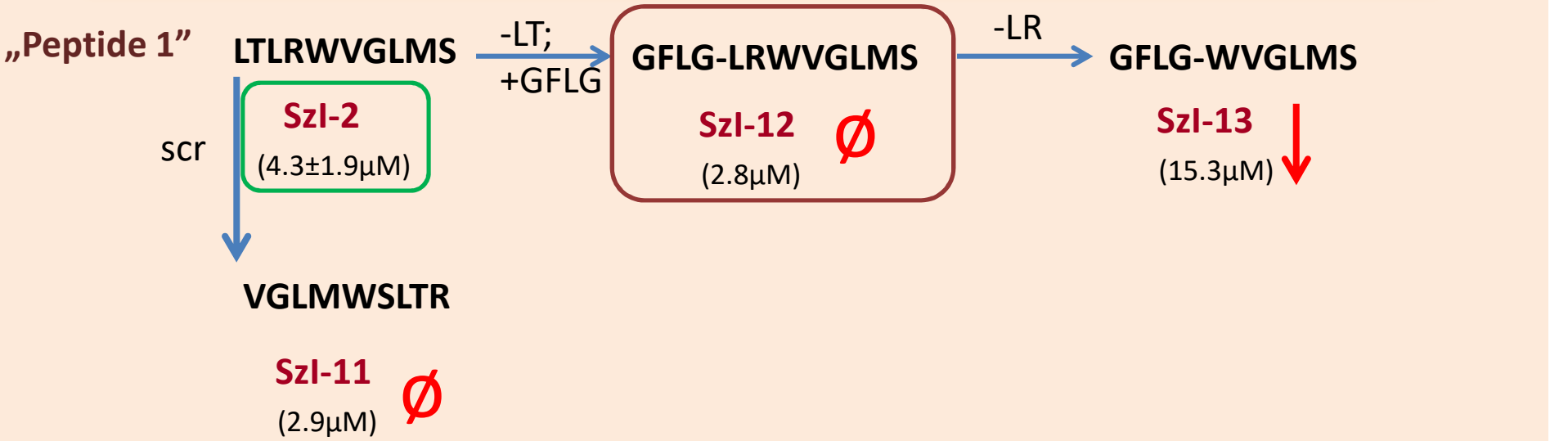
Results-preliminary data

In vitro cytostatic effect of drug containing NG2 conjugates

		IC_{50} (µM)	
		A2058	**A431**
Szl-2	**Dau=Aoa-GFLG-LTLRWVGLMS-NH_2**	**4,3±1,9**	**14.0±0.0**
Szl-11	Dau=Aoa-VGLMWSLTRL-NH_2 (scr)	2.9	3.2
Szl -12	Dau=Aoa-GFLG-LRWVGLMS-NH_2	2.8	4.8
Szl -13	Dau=Aoa-GFLG-WVGLMS-NH_2	15.3	15.7
Szl-4	**Dau=Aoa-GFLG-TAASGVRSNleH-NH_2**	**2,3±1,3**	**n.d.**
Szl -14	Dau=Aoa-GFLG-ARASNleHSTGV-NH_2 (scr)	<0.8	3.6
Szl -15	Dau=Aoa-GFLG-ASGVRSNleH-NH_2	2.3	2.9
Szl -16	Dau=Aoa-GFLG-GVRSNleH-NH_2	3.2	3.0
Dau	Dau·HCl	<0.16	Dau

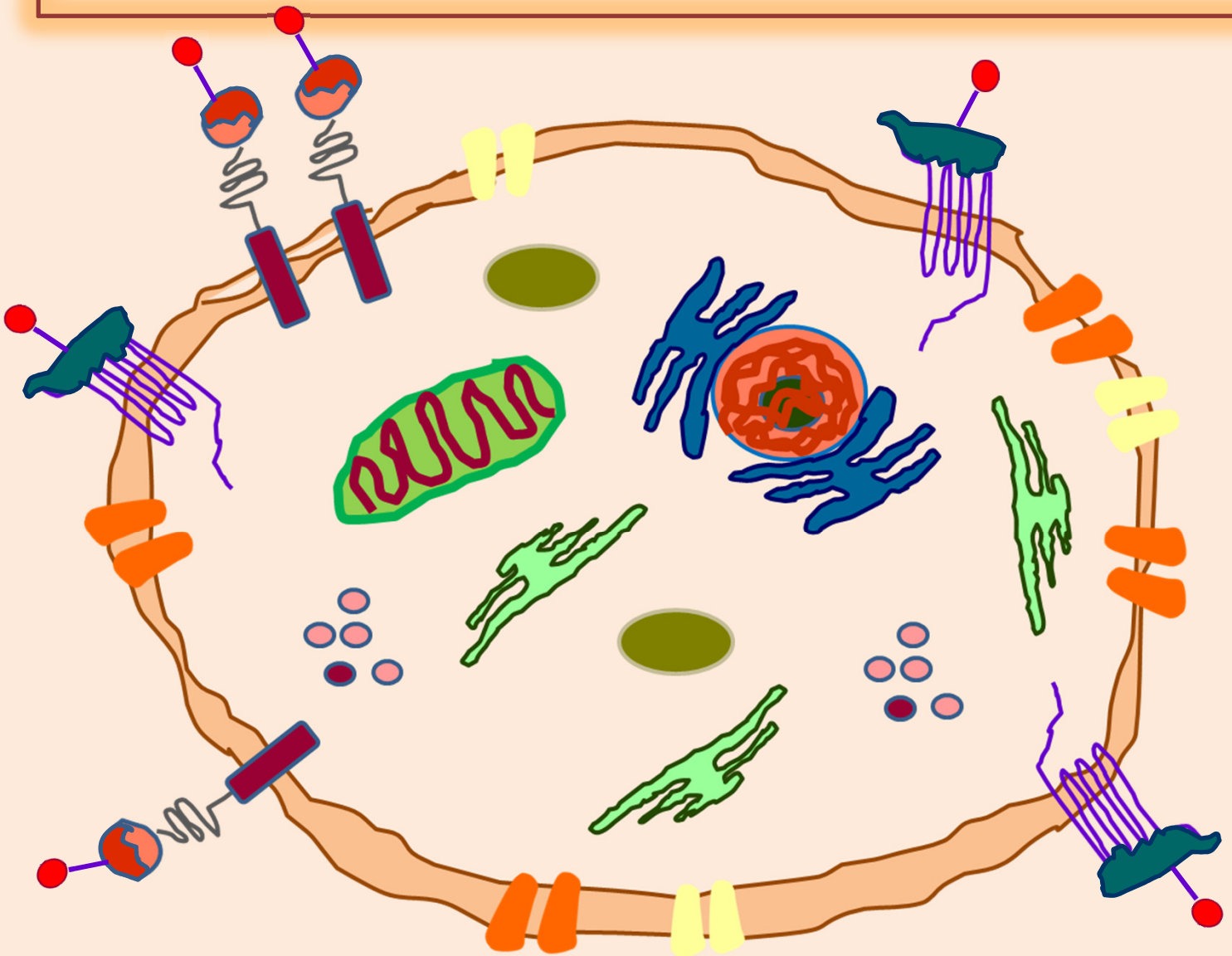
Conclusion II.

Structure-activity relationship



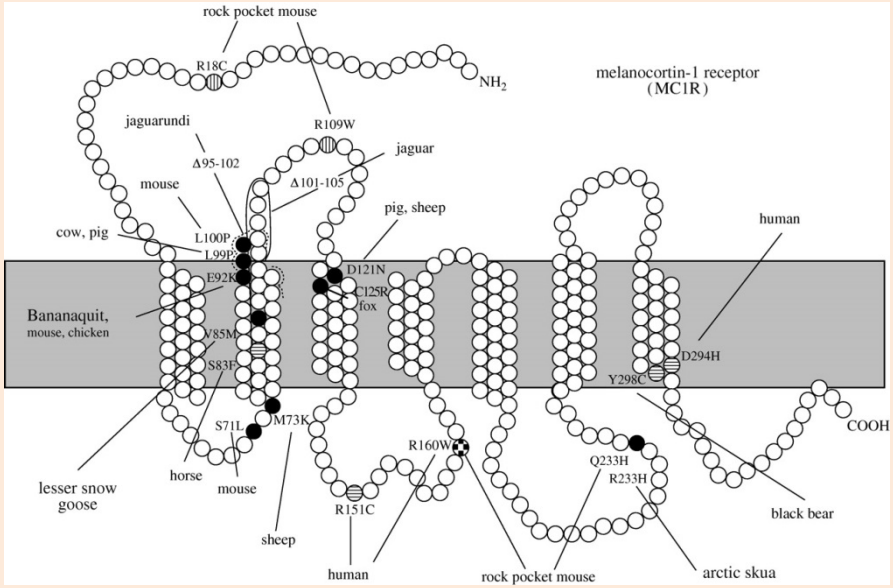
Possibilities of melanoma treatment

targeting cell surface receptors



Possibilities of melanoma treatment
Melanocortin-1 receptor (MC1R)

- GPCR; 5 subtypes with specific distribution pattern in human tissues[1,2]
- MC1R is expressed in melanocytes and melanomas[3,4]
- High level of *MC1R* gene expression is characteristic for primary and metastatic melanomas[5]
- MC1R is a highly specific marker of melanoma
- promising candidate for targeted drug delivery to melanoma cells
- MC1R ligands has specific internalization into the cells



http://rspb.royalsocietypublishing.org/content/272/1573/1633

[1]Chhajlini, V. *et al* FEBS Lett. (1992) **309**,417-420
[2]Gantz, I. *et al* J. Biol. Chem (1993) **268**, 8246-8250
[3]Schwahn, D.J. *et al* Pigment Cell Res (2001) **14**, 32-39
[4]Roberts, D.W. *et al* Pigment Cell Res (2006) **19**, 76-89
[5]Salazar-Onfray, F. *et al* Br. J. Cancer (1993) **87**, 414-422

α-MSH

α-Melanocyte Stimulating Hormone

- Ac-SYSMEHFRWGKPV-NH_2
- Produced in adenohypophysis
- regulation of skin pigmentation
- >80% of human melanoma tumor samples obtained from patients with metastatic melanoma bear α-MSH receptors
- Superagonist α-MSH analog: [Nle^4, D-Phe^7] α-MSH
  - Increased stability, resistant to enzymatic degradation
  - increased receptor affinity (<nM)
  - Ligand for targeted tumor therapy (radionucleotides, toxins, drugs, etc)

Siegrist, W. *et al.* (1989) Cancer Res, **49**, 6352–6358.
Tatro, JB *et al. (1990)* J Clin Investig, **85**,1825–1832.
Cone, RD *et al.* Ann NY Acad Sci (1993) **680**, 342–363.

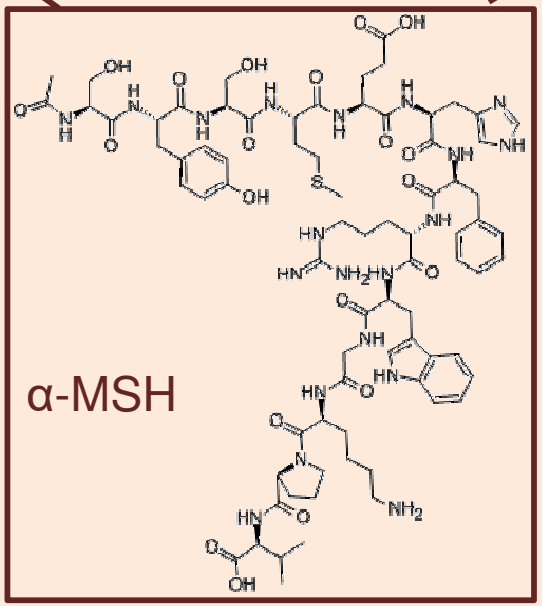
Saywer, TK *et al. (1980)* PNAS, **77**, 5754–5758.
Giblin, MF *et al. (1998)* PNAS, **95**, 12814–12818.
Morandini, R *et al. (1994)* Int J Cancer, **56**, 129–133.

Application of α-MSH in melanoma cancer

Diagnosis
Tumor imaging

Radiolabeled MSH

treatment

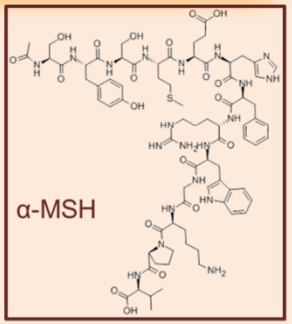


Drug-MSH conjugates

treatment

Application of α-MSH in melanoma cancer

Ala-Glu-Lys-Lys-Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp



Drug-MSH conjugates

↓

treatment

- First conjugates: Daunomycin-β-MSH,
- Melanotropin fragments have significant biological activity (nitrosurea, melphalan)
- Specific receptor recognition
- Hormon-receptor complex is rapidly internalized
- Receptor may undergo recycling

Vargha, JM *et al. (1977)* Nature, **267**, 56–58.
Garcia-Borron, J *et al. (1992)* Biochem (Life Sci. Adv), **11**, 273–277.
Orlow, SJ *et al. (1990)* J Cell Physiol, **142**, 129–136.

Application of α-MSH as targeting unit

H. Süli-Vargha; J. Botyánszky; K. Medzihradszky

α-MSH

1	2	3	4	5	6	7	8	9	10	11	12	13	
Ac-Ser-	Tyr-	Ser-	Met-	Glu-	His-	Phe-	Arg-	Trp-	Gly-	Lys-	Pro-	Val-	NH_2

Pep1	**Mel-Glu-His-Phe-Arg-Trp-Gly-OMe**
Pep2	**Nle-Glu-His-Mel-Arg-Trp-Gly-OMe**
Pep3	**Mel-Trp-Gly-Lys-Pro-Val-NH_2**
Pep4	**Mel-Lys-Pro-Val-NH_2**

Pep 1 and 2 refer to central fragments of the hormone, while Pep 3 and 4 refer to the C-terminal ones.

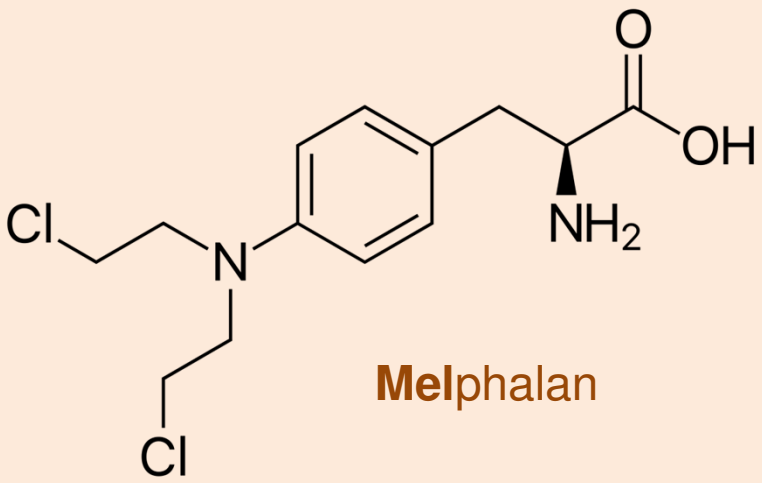
Ac-SYSMEHFRWGKPV-NH_2

↓

E^5HFRWG^{10}

TABLE I – COMPARISON OF IC_{50} EXPRESSED AS μG/ML MELPHALAN EQUIVALENT FOR ALL THE CONJUGATES AND THE FREE DRUG ON 3 DIFFERENT CELL LINES

	IC_{50} μg/ml		
	HBL melanoma	F-NBB fibroblasts	Me-180 carcinoma
Pep 1	0.9	6.0	5.2
Pep 2	21.3	> 125	> 125
Pep 3	4.8	45.0	4.6
Pep 4	2.2	45.0	5.2
Melph	0.40	0.9	1.5

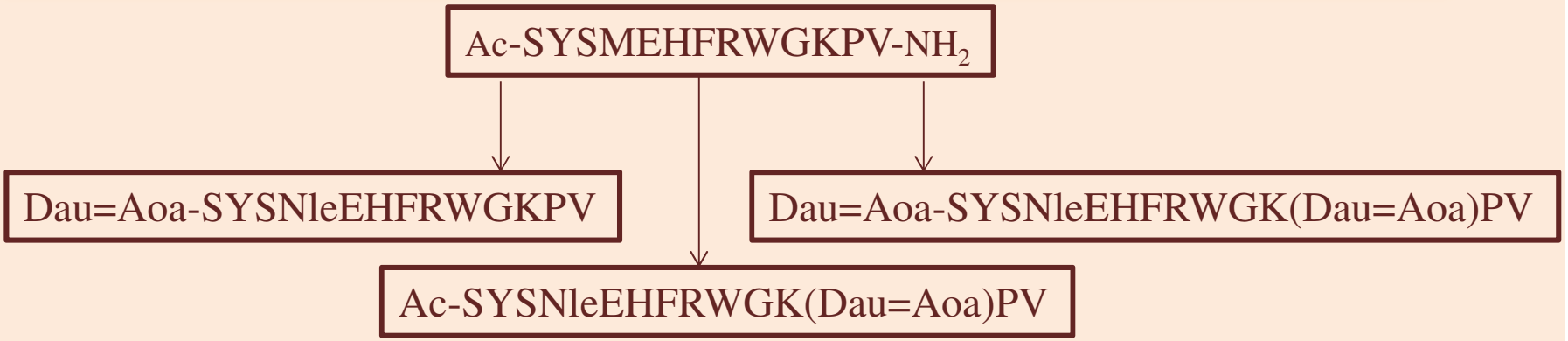


Melphalan

- Central fragment containing conjugate has selective and specific cytotoxic effect
- The effect is mediated by MC1R

Morandini, R. *et al* Int. J. Cancer (1994) **56**, 129-133

Chemical characterization of drug containing a-MSH conjugates



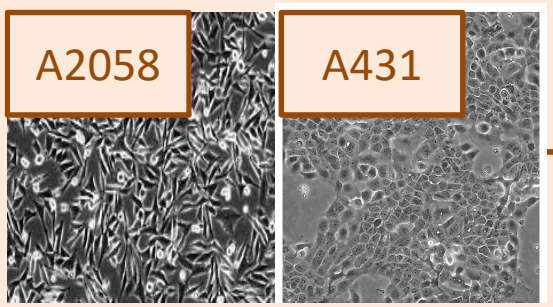
Code	Conjugates	t_R (min)[a]	M_{calc}	M_{meas}[b]
SzI-7	Dau=Aoa-SYSNleEHFRWGKPV-NH_2	12.8	2185.8	2186.1
SzI-8	Ac-SYSNleEHFRWGK(Dau=Aoa)PV-NH_2	13.1	2228.0	2228.5
SzI-9	Dau=Aoa-SYSNleEHFRWGK(Dau=Aoa)PV-NH_2	13.0	2768.0	2768.4

[a] Analitical RP-HPLC, Agilent Eclipse XDB C8, 5 µm, 80Å, 4.6 x 150 mm, HPLC coloumn, gradient: 5% B, 2 min; 5-100% B, 20 min.

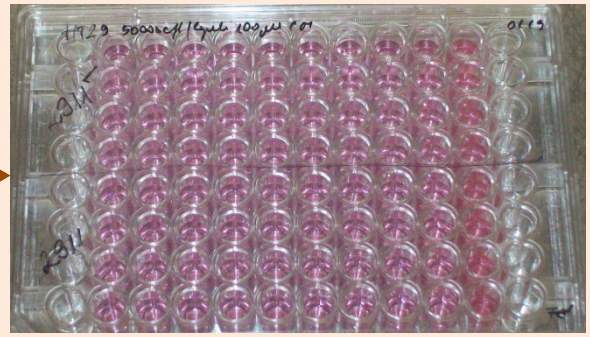
[b] Bruker Daltonics Esquire 3000plus (Bremen, Germany) ion trap mass spectrometer. Spectra were acquired in the 50–2000 m/z range

Determination of *in vitro* cytostatic effect of drug containing α-MSH conjugates

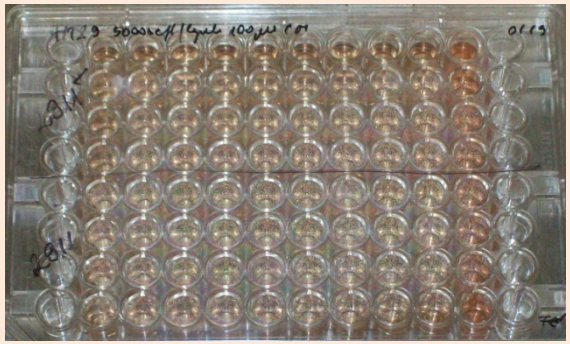
MTT-assay



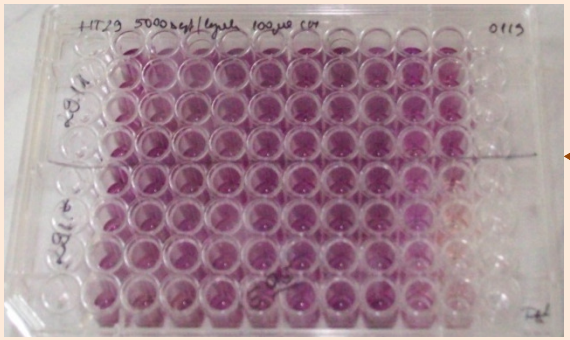
(24h, 37°C)



Treatment
(24h, 37°C, CM,concentration:
0.8 – 100 µM;
Washing, culturing in FCSM, 48h, , 37°C)



MTT-assay (3.5h, 37°C)



Determination of IC_{50} values

In vitro efficacy of daunomicin containing peptide conjugates

		IC_{50} (μM)	
		A2058	A431
SzI -7	Dau=Aoa-SYSNleEHFRWGKPV-NH_2	9.8±5.4	25.0±11.2
SzI -8	Ac-SYSNleEHFRWGK(Dau=Aoa)PV-NH_2	3.2±0.4	8.8±5.9
SzI -9	Dau=Aoa-SYSNleEHFRWGK(Dau=Aoa)PV-NH_2	3.0±0.8	16.5±1.6
Dau	Dau·HCl	<0.16	0.5±0.4

		IC_{50} (μM)		
		B16	M24	WM983b
SzI -7	Dau=Aoa-SYSNleEHFRWGKPV-NH_2	2.9±0.6	12.8±1.6	9.9±1.5
SzI -8	Ac-SYSNleEHFRWGK(Dau=Aoa)PV-NH_2	2.8±0.7	11.5±0.4	7.9±0.7
SzI -9	Dau=Aoa-SYSNleEHFRWGK(Dau=Aoa)PV-NH_2	2.0±0.7	11.0±0.8	3.6±0.2

Conclusion III.

Structure-activity relationship

Dau=Aoa-SYSNleEHFRWGKPV-NH_2

Szl-7
(9.8±5.4μM)

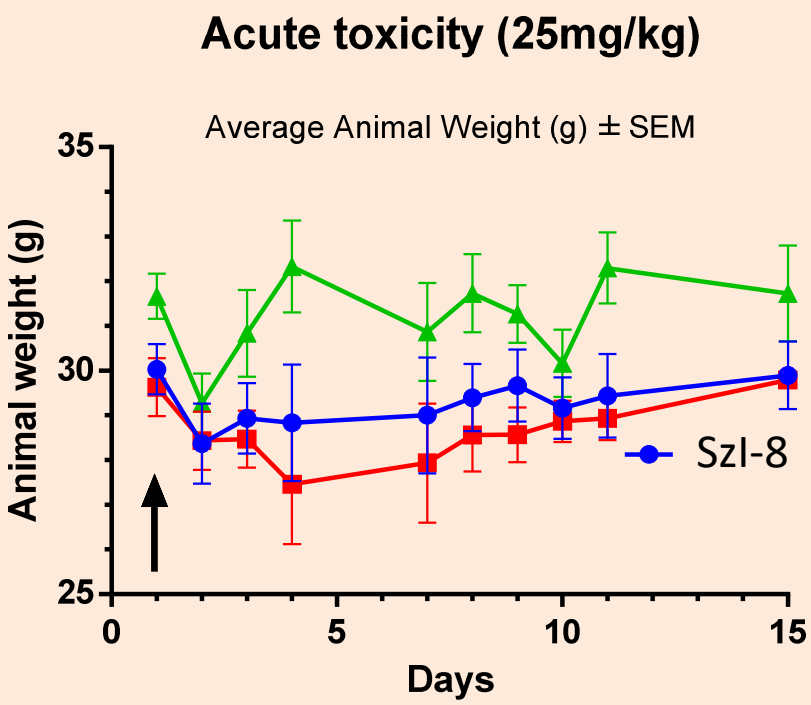
Ac-SYSNleEHFRWGK(Dau=Aoa)PV-NH_2

Szl-8
(3.2±0.4μM)

Dau=Aoa-SYSNleEHFRWGK(Dau=Aoa)PV-NH_2

Szl-9
(3.0±0.8μM)

In vivo antitumor activity of drug containing α-MSH conjugates



Conditions:
adult BALB/c male mice (28-32g) ;
(i.p.) administration;
25 mg DAU content/kg;
3 mice/ group
The toxicity was evaluated on the basis of life span, behavior and looking of the mice, and body weight.
Parameters were followed for 14 days.

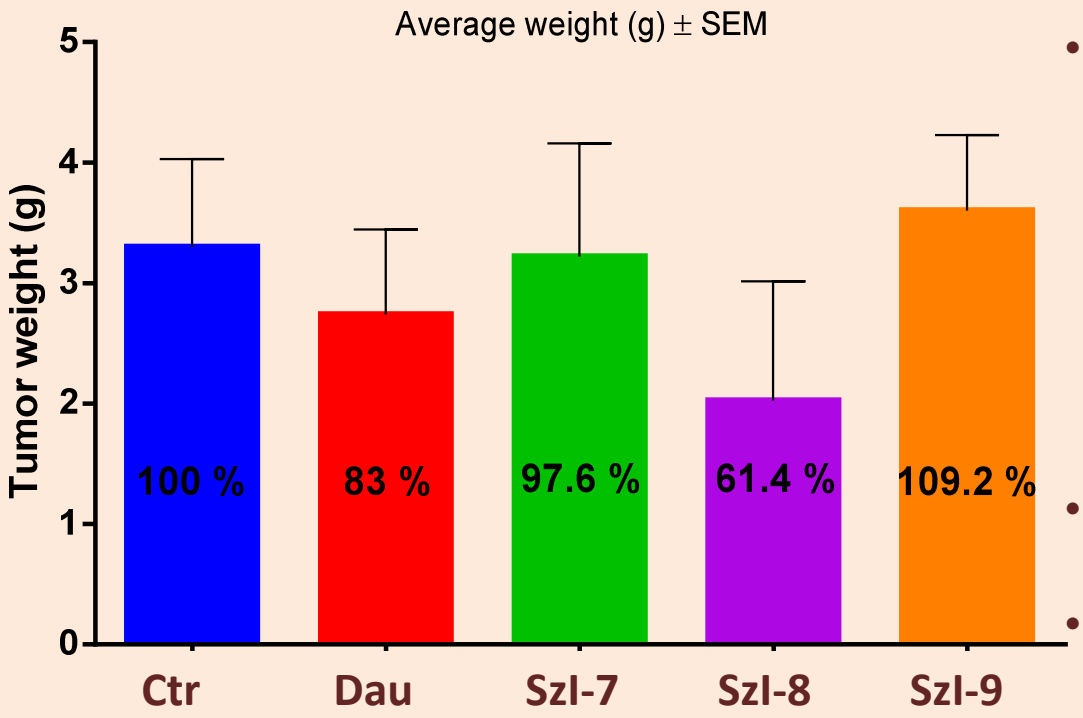
After 14 days following, significant change in body weight, behaviour, and also in general looking was not observed.

↓

not toxic for the animals →that can be further investigated their antitumor activity *in Vivo*.

In vivo antitumor activity of drug containing α-MSH conjugates

Antitumor activity of conjugates SzI-7,-8,-9 and free Dau in s.c. B16 melanoma model

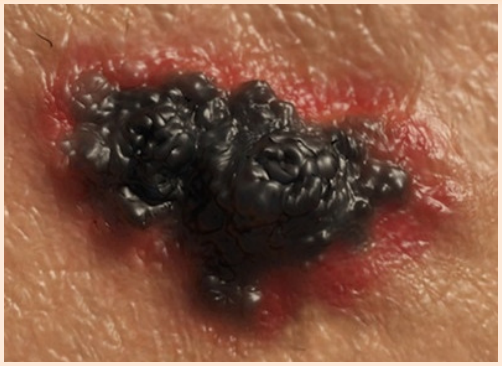


- B16 (s.c.) injected into C57BL/6 male mice (20-28g), 7 animal/group
- i.p. administration.
- doses:
  - control group (solvent);
  - free DAU group (1 mg/kg, treatment on day 9 and 17);
  - SZI-7, SZI-8 and SZI-9 groups (10 mg/kg DAU content, treatment on day 9, 13, 15, 17 after cells inoculation).
- Termination: 20 days after cell inoculation,
- Determination: Animal weight and tumor volumes

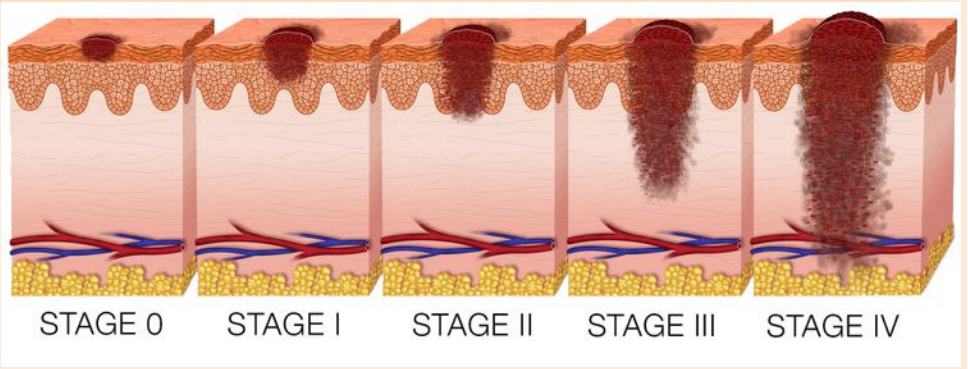
SZI-8> SZI-7>> SZI-9

Conjugate SZI-8 showed higher anti-tumor activity in comparison with free Dau

Summary



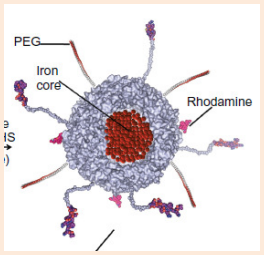
localization

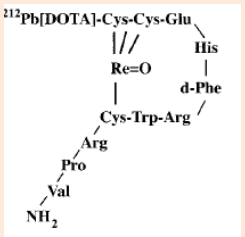


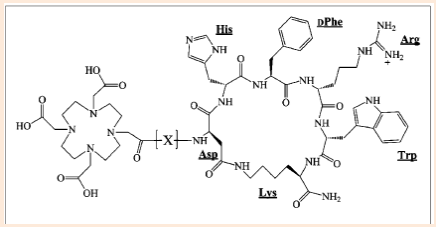
Rapid progression

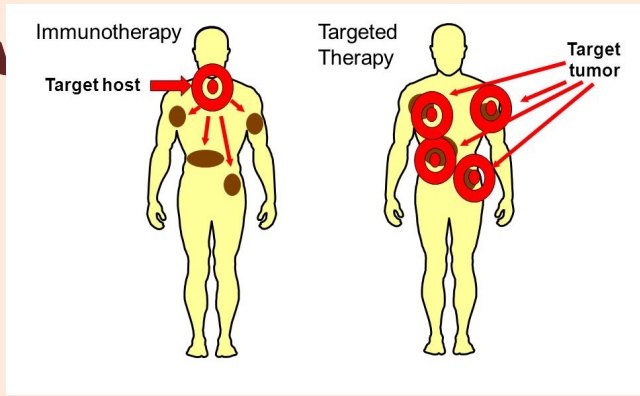
Melanoma

Drug — Peptide









TIME

Thank You for Your Attention!