NANOTECNOLOGIE PER LO STUDIO E LA CURA DEL CERVELLO

MARIO NEGRI
1961 - 2011

Tre punti chiave:

1. POTENZIALE DELLE NANOTECNOLOGIE IN AMBITO DIAGNOSTICO E TERAPEUTICO

2. COSA INTENDIAMO PER NANOMEDICINA

3. COME SVILUPPARE NANOFARMACI

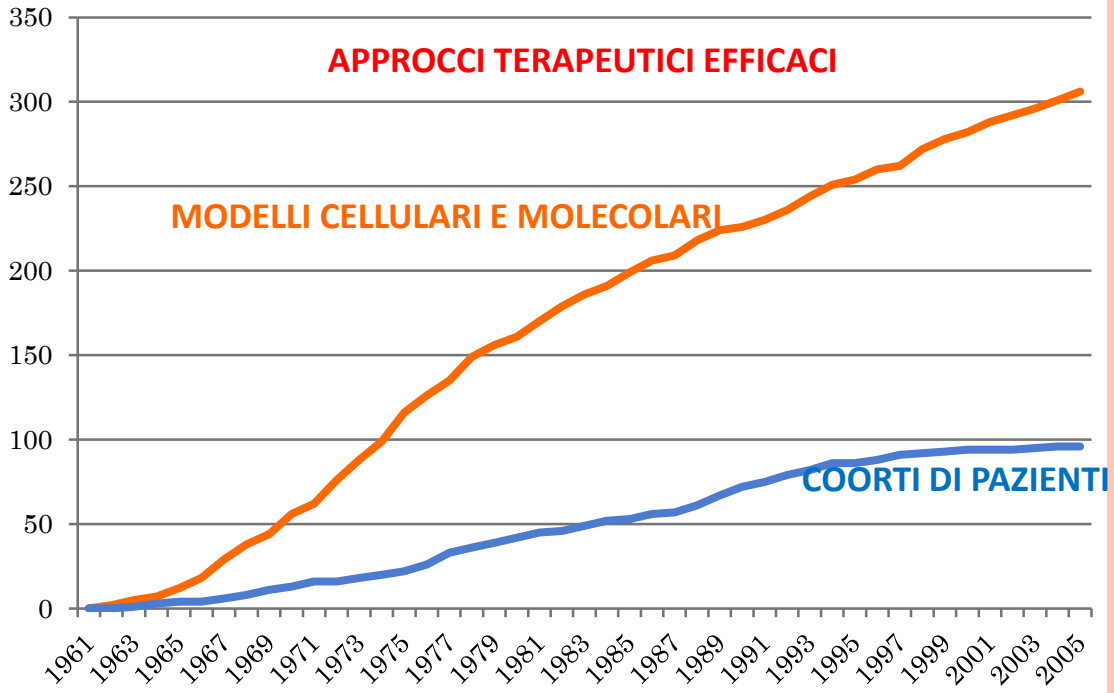
RICERCA DI BASE Vs PRATICA CLINICA: UNA FORBICE SEMPRE PIU' APERTA

MALATTIE NEURODEGENERATIVE (PARKINSON, ALZHEIMER, STROKE)

TUMORI SOLIDI, LEUCEMIE

PATOLOGIE CARDIOVASCOLARI

PATOLOGIE AUTOIMMUNI, DIABETE



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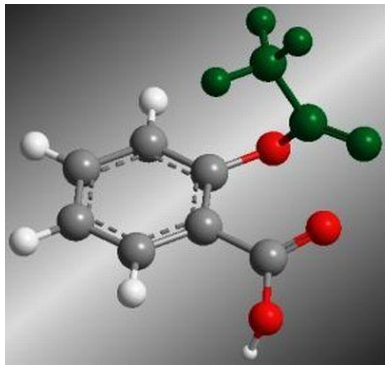
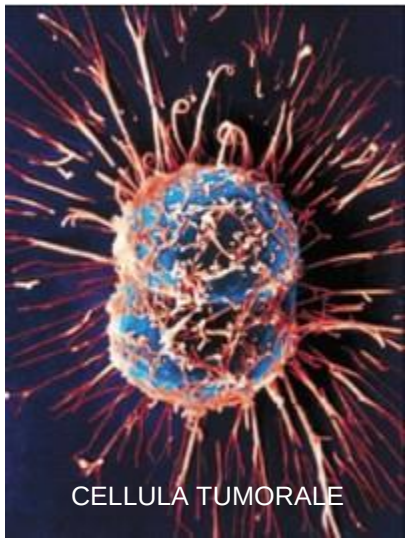
DALLA CELLULA…..

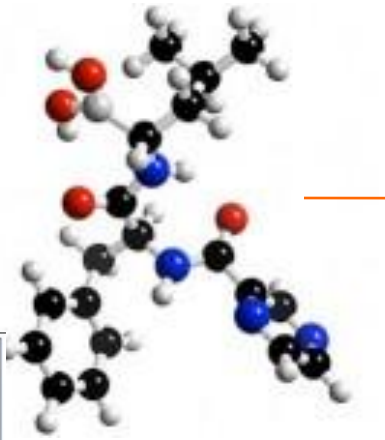


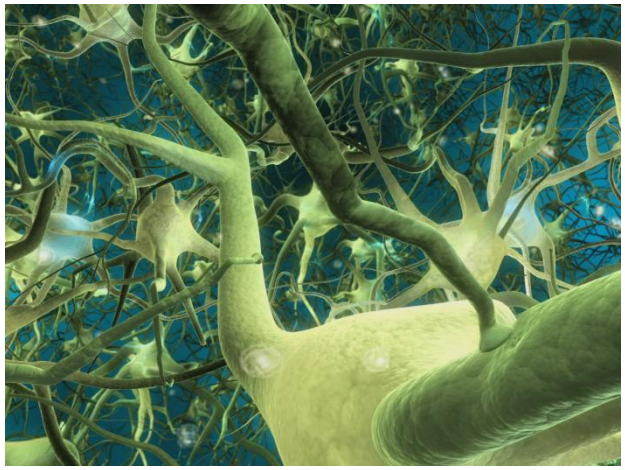


Jan Bruegel il vecchio "Paradiso terrestre"

TOSSICITA'

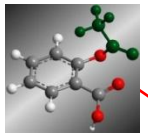
AGENTE TERAPEUTICO → BERSAGLIO CELLULARE →VALUTAZIONE EFFETTO

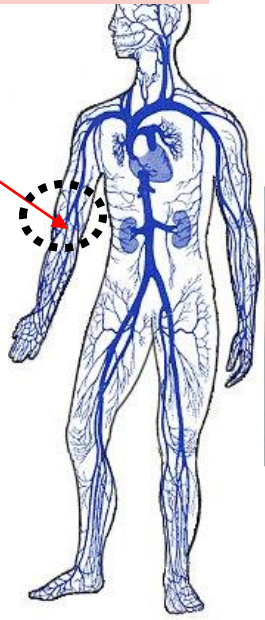




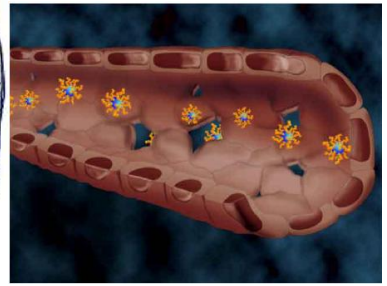
PROTEZIONE

MARIO NEGRI
ISTITUTO DI RICERCHE FARMACOLOGICHE
1961 - 2011

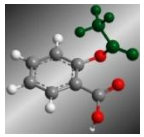




.....ALL'UOMO



"PER ME SI VA NE LA CITTA' DOLENTE,
PER ME SI VA NE L'ETERNO DOLORE,
PER ME SI VA TRA LA PERDUTA GENTE.
GIUSTIZIA MOSSE IL MIO ALTO FATTORE:
FECEMI LA DIVINA POTESTATE,
LA SOMMA SAPIENZA E 'L PRIMO AMORE.
DINANZI A ME NON FUR COSE CREATE
SE NON ETERNE, E IO ETERNA DURO.
LASCIATE OGNI SPERANZA, VOI CH'ENTRATE."
(*Inferno*, Canto III, vv. 1-9)



LEGAME PROTEINE PLASMATICHE
FAGOCITOSI
INATTIVAZIONE
TRASPORTO FEGATO, MILZA E RENI

BIODISTRIBUZIONE ASPECIFICA

METABOLISMO E INATTIVAZIONE
ESCREZIONE
ACCUMULO E TOSSICITA'

SOMMINISTRAZIONE SISTEMICA

BARRIERE BIOLOGICHE

EMATO-ENCEFALICA
BARRIERA TUMORALE
MATRICE EXTRACELLULARE

DOSE EFFICACE / DOSE SOMMINISTRATA
BASSO

TOSSICITA' SISTEMICA
ELEVATA

E COME PUO' AIUTARCI NANOTECNOLOGIA?

PARTIAMO DA UNA FREDDA DEFINIZIONE.....

Voce | Discussione | Leggi | Modifica | Visualizza cronologia

Nanomedicina

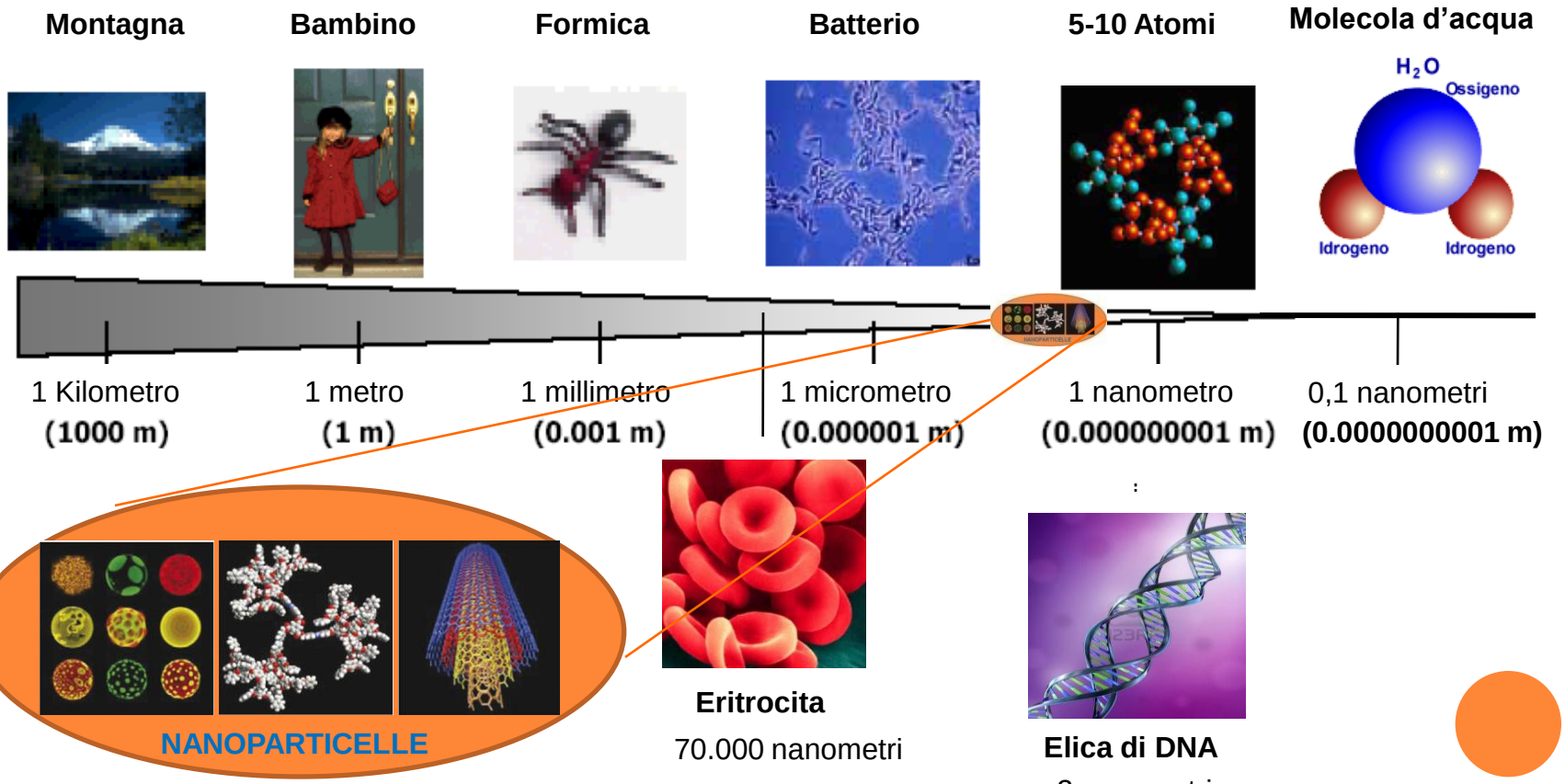
Da Wikipedia, l'enciclopedia libera.

WIKIPEDIA
L'enciclopedia libera

La nanomedicina è l'applicazione medica delle possibilità derivanti dalle nanotecnologie. Essa si occupa quindi di tutte quelle conoscenze e quelle tecnologie che abbiano un utilizzo medico nell'ordine di grandezza dei nanometri (1-150nm). Lavorando a tali dimensioni la nanotecnologia altera la tradizionale distinzione tra BIOLOGIA, CHIMICA, FISICA E MEDICINA.

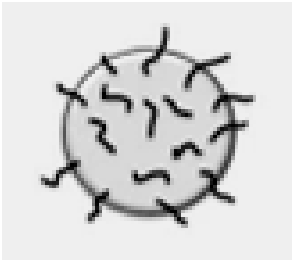
"NANO", LA "TECNOLOGIA DEL PICCOLISSIMO":

1 nanometro (nm) è uguale a **un miliardesimo** (0.000000001) **di metro**



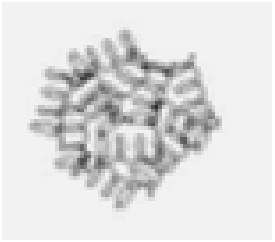
LA NANOMEDICINA NASCE DALL'UNIONE DI.....

1. NANOSTRUTTURE

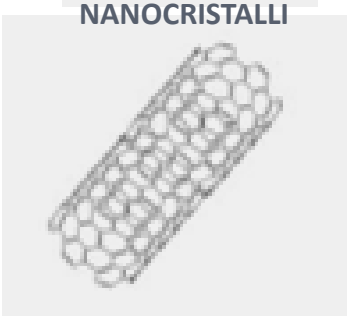


PARTICELLE INORGANICHE

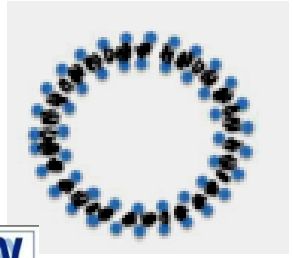
NANOCRISTALLI



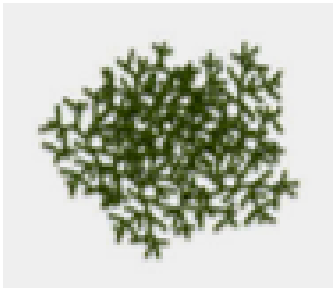
PARTICELLE POLIMERICHE



NANOTUBI



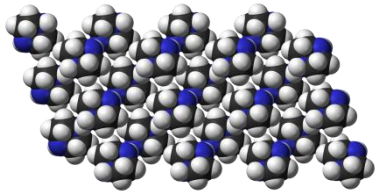
PARTICELLE LIPIDICHE (LIPOSOMI)



NANODENDRIMERI

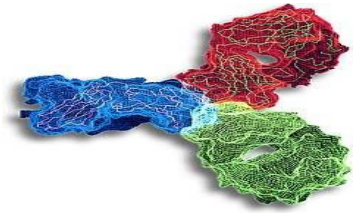
.....CON

2. AGENTI CHIMICI E/O BIOLOGICI

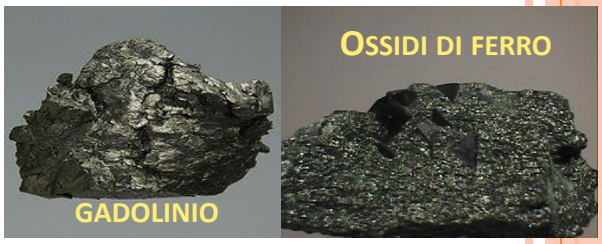


COMPOSTI INORGANICI (FARMACI TRADIZIONALI)

SONDE FLUORESCENTI

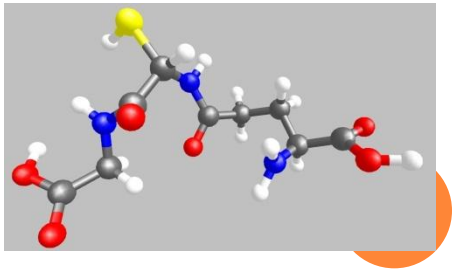


ANTICORPI



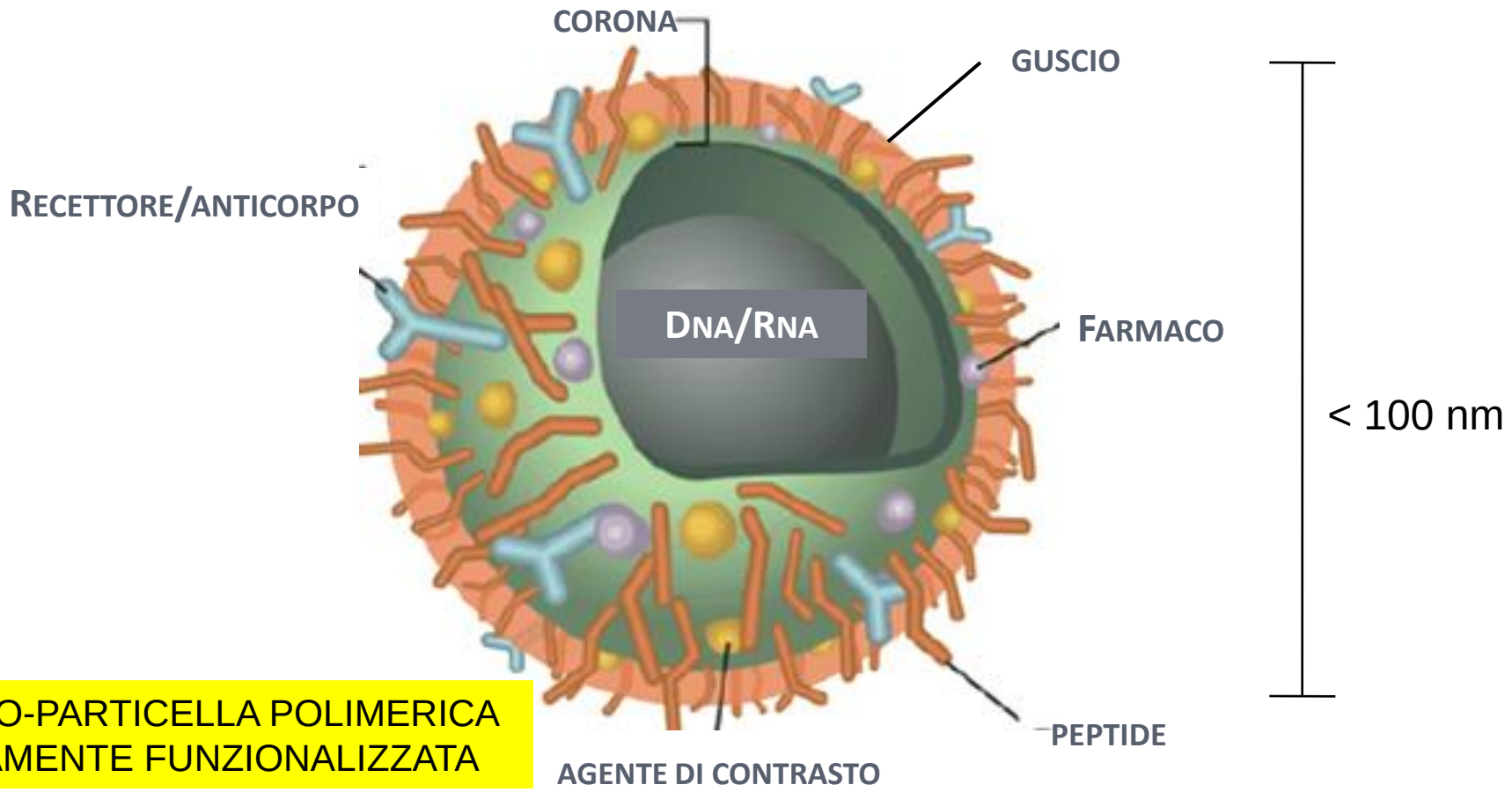
AGENTI DI CONTRASTO

ACIDI NUCLEICI



PEPTIDI

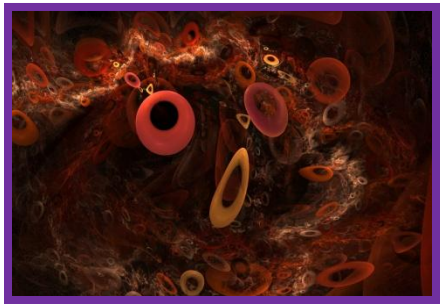
AL FINE DI GENERARE UNA STRUTTURA “COMPLESSA” :



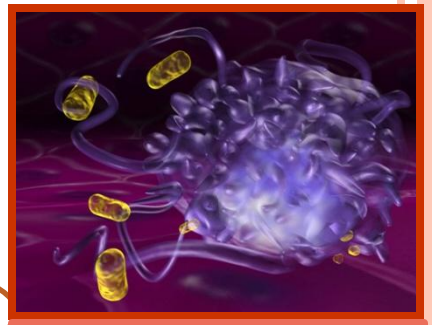
NANO-PARTICELLA POLIMERICA ALTAMENTE FUNZIONALIZZATA

CHE POSSA ASSOCIARE PROPRIETA’ TERAPEUTICHE E DIAGNOSTICHE IN UN VOLUME NANOMETRICO

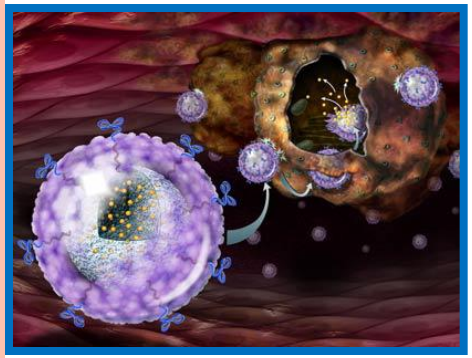
STABILITA' IN CIRCOLO E PRESERVANO LA MOLECOLA DA DIGESTIONE ENZIMATICA

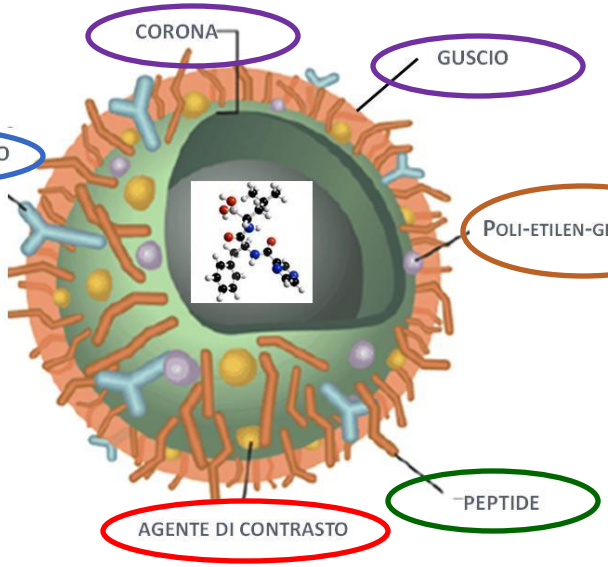


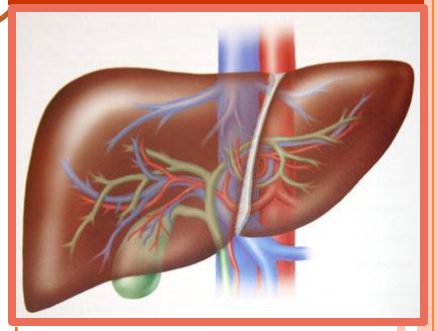
LEGAME CON LE PROTEINE PLASMATICHE E LA FAGOCITOSI



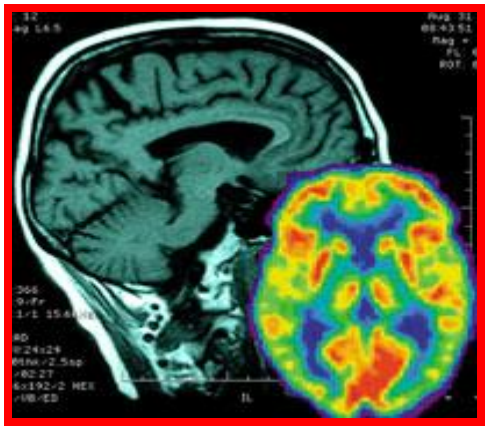
SPECIFICITA' VERSO BERSAGLI CELLULARI TERAPEUTICI



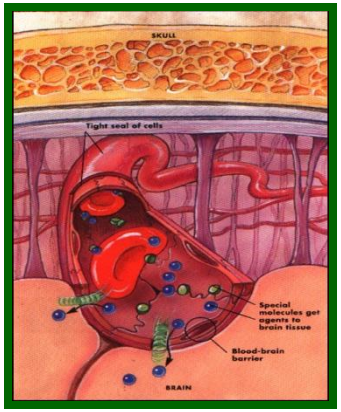




TRACCIABILITA' DEL FARMACO NEI VARI ORGANI E NEL TEMPO



ACCUMULO E METABOLISMO EPATICO



PASSAGGIO ATTRAVERSO BARRIERE BIOLOGICHE SPECIFICH

MARIO NEGRI ISTITUTO DI RICERCHE FARMACOLOGICHE 1961 - 2011

Targeted nanoparticle enhanced proapoptotic peptide as potential therapy for glioblastoma

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Edited by Mark Groudine, Fred Hutchinson Cancer Research Center, Seattle, WA, and approved September 16, 2011 (received for review September 9, 2011)

Antiangiogenic therapy can produce transient tumor regression in glioblastoma (GBM), but no prolongation in patient survival has been achieved. We have constructed a nanosystem targeted to tumor vasculature that incorporates three elements: (*i*) a tumor-homing peptide that specifically delivers its payload to the mitochondria of tumor endothelial cells and tumor cells, (*ii*) conjugation of this homing peptide with a proapoptotic peptide that acts on mitochondria, and (*iii*) multivalent presentation on iron oxide nanoparticles, which enhances the proapoptotic activity. The iron oxide component of the nanoparticles enabled imaging of GBM tumors in mice. Systemic treatment of GBM-bearing mice with the nanoparticles eradicated most tumors in one GBM mouse model and significantly delayed tumor development in another. Coinjecting the nanoparticles with a tumor-penetrating peptide further enhanced the therapeutic effect. Both models used have proven completely resistant to other therapies, suggesting clinical potential of our nanosystem.

angiogenesis | apoptosis | tumor targeting | tumor treatment

Tumor blood vessels have become an important therapeutic target. As a tumor grows, the blood vessels grow with it, and this growth primarily takes place through angiogenesis (1, 2). Therefore, inhibiting angiogenesis has become a mainstream therapeutic strategy. The special features of tumor vasculature also enable another strategy, homing-based (synaphic) delivery of drugs (3). Tumor blood vessels express various cell surface and extracellular matrix proteins that normal vessels do not express or do so at much lower levels than tumor vessels (1, 3). These specific vascular markers are readily available to bind circulating ligands, such as peptides and antibodies (4–6). Drugs attached to such ligands become concentrated in tumor tissue, thereby improving efficacy and reducing the exposure of normal tissues (3).

Vascular markers can be explored in an unbiased manner by in vivo screening of phage libraries that display random peptide sequences (7). The CGKRK (Cys-Gly-Lys-Arg-Lys) peptide we used in this study was identified by screening for peptides homing to epidermal tumors in mice (8). Intravenous injected CGKRK recognizes the vessels in most tumors but not those in normal tissues (8).

The α-helical amphipathic peptide $_D$[KLAKLAK]$_2$ was originally designed as a synthetic antibacterial peptide that disrupts the bacterial cell membrane but is less toxic to eukaryotic cells (9). However, when internalized into eukaryotic cells, $_D$[KLAKLAK]$_2$ disrupts the mitochondrial membrane, which is similar to bacteria membranes, and initiates apoptotic cell death (10). Conjugating $_D$[KLAKLAK]$_2$ with homing peptides has produced compounds that specifically accumulate at the homing target, causing cell death (11–15). Here we made a tumor-homing $_D$[KLAKLAK]$_2$ compound by conjugating $_D$[KLAKLAK]$_2$ to CGKRK. $_D$[KLAKLAK]$_2$, however, is a highly toxic compound, even when specifically targeted to tumors (11, 13). Administering toxic drugs in a nanoparticle formulation can reduce toxicity. Examples include paclitaxel–albumin nanoparticles [Abraxane (16)] and doxorubicin liposomes [Doxil (17)], both of which are in clinical use. Other advantages of nanoparticles include the fact that compounds coupled onto their surface are presented in a multivalent fashion, which increases the binding efficiency at the target. Further, multiple functions can be built into a nanoparticle.

Here we assembled a multifunctional theranostic nanoparticle in which the CGKRK peptide provides the targeting function that takes the nanoparticles to tumor vascular cells and into their mitochondria. The nanoparticle uses the mitochondria-targeted $_D$[KLAKLAK]$_2$ peptide as the drug and iron oxide as a diagnostic component for MRI. Finally, we combined the nanoparticles with the tumor-penetrating peptide iRGD (18, 19), which enhances the penetration of the nanoparticles into the extravascular tumor tissue. The activity of this nanosystem was tested in one of the most difficult to treat tumors, glioblastoma (GBM). Despite a multimodality treatment approach, which includes surgery, irradiation, and chemotherapy, the median survival in this cancer is only 12 mo (20). Thus, more effective treatments are desperately needed.

Results

CGKRK Peptide Homes to Brain Tumors and Colocalizes with Mitochondria in Cells. We tested a number of previously identified tumor-homing peptides for homing to GBM tumors in mice after an i.v. injection (21, 22). Rhodamine (Rd)-labeled CGKRK peptide (8) strongly accumulated in GBM tumors but not in normal brain or other normal tissues, with the exception of the kidneys, where peptides are excreted (Fig. 1*A* and Fig. S1).

CGKRK is internalized into the target cells and can take a payload with it (8). FAM-CGKRK colocalized with a mitochondrial marker in human umbilical vein endothelial cells (HUVEC) and U87, human GBM cells (Fig. 1*B*). CGKRK bound to mitochondria isolated from mouse liver (Fig. 1*C*). The binding was inhibited by unlabeled CGKRK but not a control peptide with a similar structure (CREKA), demonstrating the specificity of the mitochondria binding. CGKRK-displaying phage showed 80-fold higher binding to the isolated mitochondria than a control phage, further supporting the notion that mitochondria are the primary subcellular target of CGKRK.

Intratumoral Distribution of Iron Oxide Nanoworms Coated with CGKRK$_D$(KLAKLAK)$_2$. The mitochondrial localization of CGKRK suggested a way of improving the delivery of a proapoptotic peptide, $_D$[KLAKLAK]$_2$, which acts on mitochondria (10). We set up a targeting system that consists of the $_D$[KLAKLAK]$_2$ peptide as a drug and CGKRK as a targeting element, coupled to iron oxide nanoparticles, dubbed "nanoworms" (NWs) because of their

Author contributions: L.A., D.F.-M., V.R.K., K.N.S., I.M.V., and E.R. designed research; L.A., D.F.-M., V.R.K., L.R., O.M.G., and E.R. performed research; L.A., D.F.-M., V.R.K., and I.M.V. contributed new reagents/analytic tools; L.A., D.F.-M., V.R.K., K.N.S., O.M.G., R.F.M., I.M.V., and E.R. analyzed data; and L.A., D.F.-M., and E.R. wrote the paper.

Conflict of interest statement: E.R., V.R.K., and K.N.S. are shareholders in CendR Therapeutics Inc., which has rights to some of the technology described in this paper.

This article is a PNAS Direct Submission.

[1]L.A. and D.F.-M. contributed equally to this work.

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PUBBLICAZIONE IN UNA RIVISTA BIOMEDICA DI NOTEVOLE IMPORTANZA

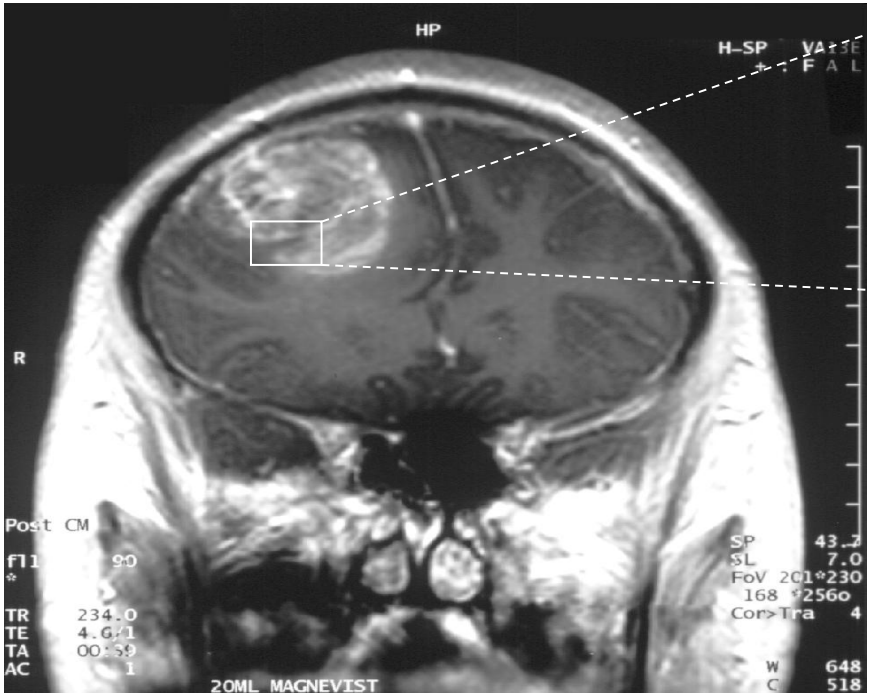
NANOPARTICELLE POLIFUNZIONALI COME POTENZIALI AGENTI TERAPEUTICI NEI TUMORI CEREBRALI

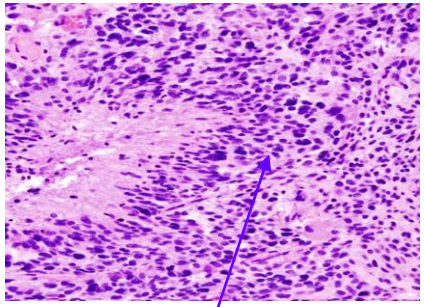
IL GLIOBLASTOMA MULTIFORME E' IL PIU' COMUNE E PIU' AGGRESSIVO TUMORE CEREBRALE UMANO

LA SOPRAVVIVENZA MEDIA DALLA DIAGNOSI VARIA DAI 3 MESI AI 2 ANNI

LA PROGNOSI E' PER OLTRE IL 90% DEI CASI INFAUSTA

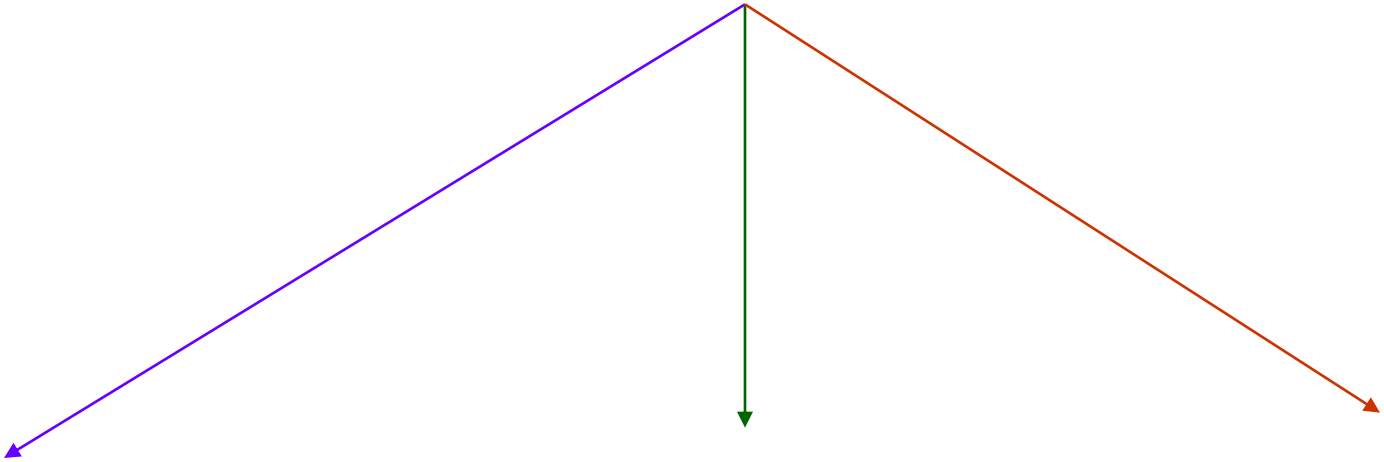
LA GUARIGIONE E' PER LA MAGGIOR PARTE DEI CASI DOVUTA AD UNA RESEZIONE CHIRURGICA COMPLETA O A UNA MORTE SPONTANEA DELLA MASSA





Cellule tumorali che invadono il cervello

PERCHE' LA TERAPIA NON E' EFFICACE?

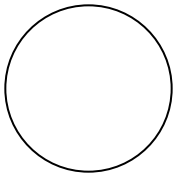


LE CELLULE DI GLIOBLASTOMA SONO MOLTO RESISTENTI AI CHEMOTERAPICI

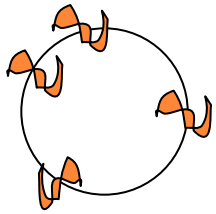
IL CERVELLO E' UN SISTEMA MOLTO DELICATO E RICHIEDE UNA TERAPIA MOLTO SPECIFICA

LA BARRIERA EMATOENCEFALICA NON FACILITA IL PASSAGGIO DI MOLTI FARMACI ATTIVI *IN VITRO*

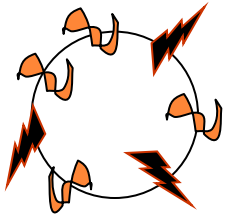
COME PUO' CONTRIBUIRE LA NANOTECNOLOGIA?



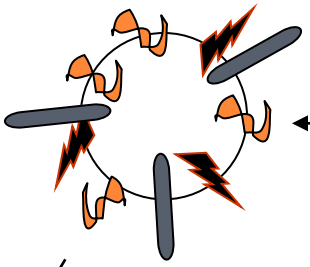
NANOPARTICELLA POLIMERICA
BIODEGRADABILE
~ 50 NM



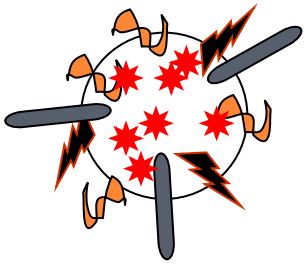
1° PEPTIDE CHE RICONOSCE SPECIFICATAMENTE I VASI DEL TESSUTO TUMORALE E VI SI LEGA IRREVERSIBILMENTE

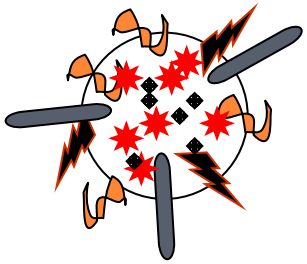


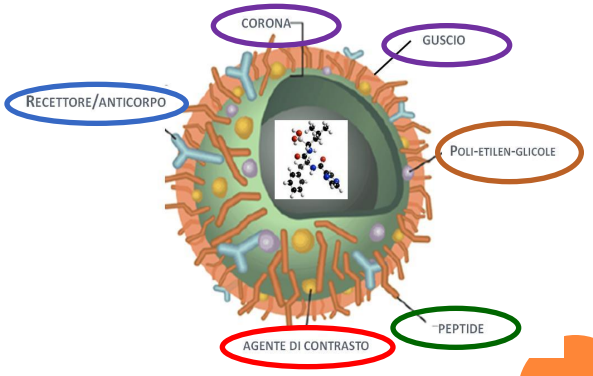
2° PEPTIDE CHE PROVOCA TOSSICITA' SULLE CELLULE TUMORALI (GIA' TESTATO *IN VITRO*)



MOLECOLA CHE RIDUCE LA FAGOCITOSI DA PARTE DEI MACROFAGI CIRCOLANTI







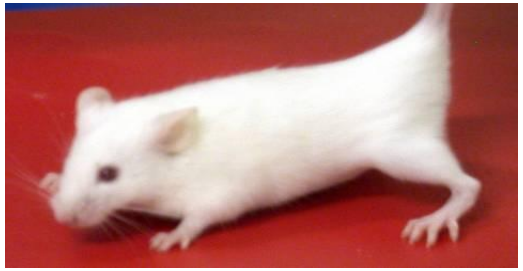
OMPOSTO FLUORESCENTE (RODAMINA) CHE CONSENTE DI INDIVIDUARE LE NP DOPO L'INIEZIONE SISTEMICA

AGENTE DI CONTRASTO (OSSIDO DI FERRO) CHE PERMETTE DI VALUTARE LA DISTRIBUZIONE DELLE NP IN TEMPO REALE (MRI)

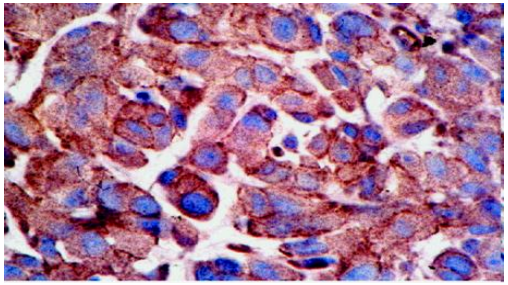
PROCEDURA SPERIMENTALE

IMPIANTO TUMORE

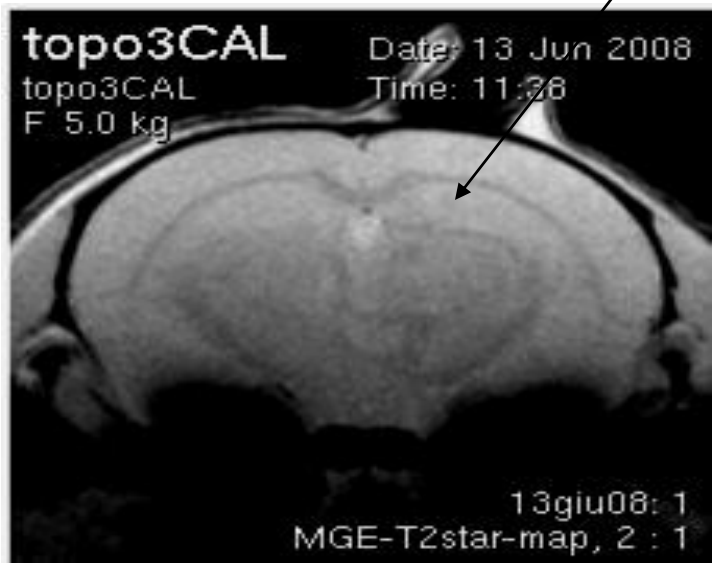
TOPI IMMUNODEPRESSI
(CRESCITA DI CELLULE TUMORALI UMANE SENZA RIGETTO)

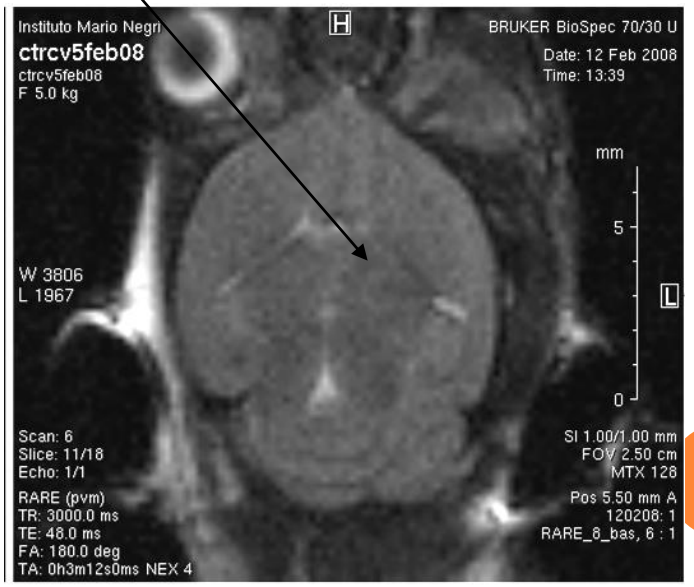


Cellule di glioblastoma umanoU87-MG
RESE VISIBILI MEDIANTE ESPRESSIONE DI UNA PROTEINA FLUORESCENTE



Impianto delle cellule in una regione specifica del cervello
(IPPOCAMPO)





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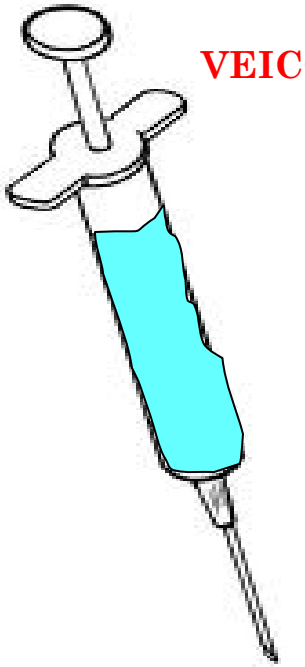
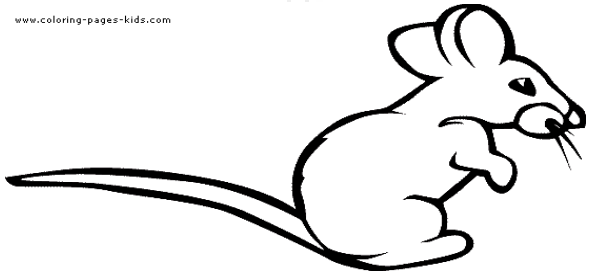
TRATTAMENTO ANIMALI

A

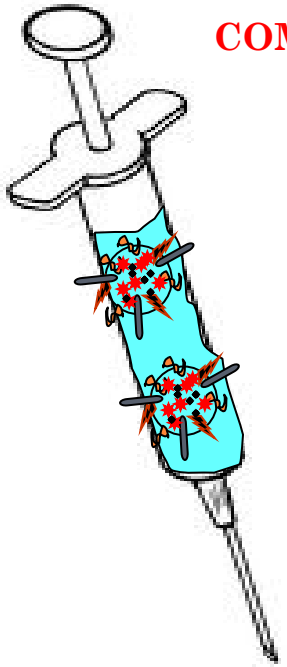
VEICOLO

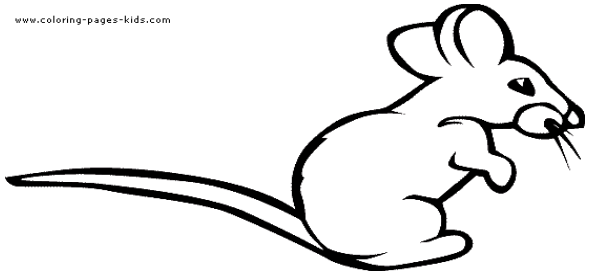




B

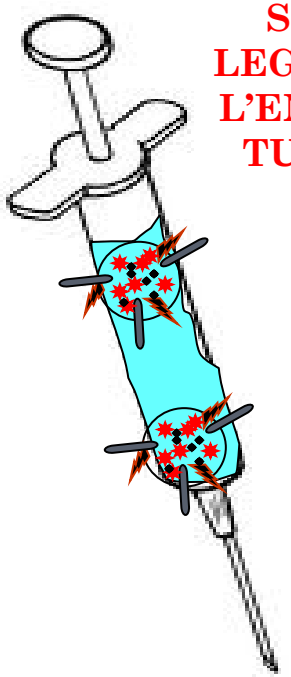
NPs COMPLETE

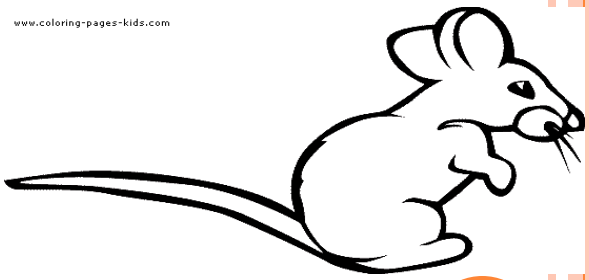




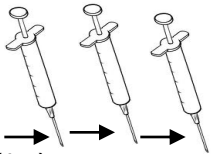
C

NPs SENZA IL LEGANTE PER L'ENDOTELIO TUMORALE





IMPIANTO TUMORE → CRESCITA TUMORE (21 gg) → **INIZIO TRATTAMENTO** (1 g)



(OGNI 7 gg)

A B C

(ANALISI SOPRAVVIVENZA)

(ANALISI ISTOLOGICHE)

Obiettivi principali (outcomes primari)!

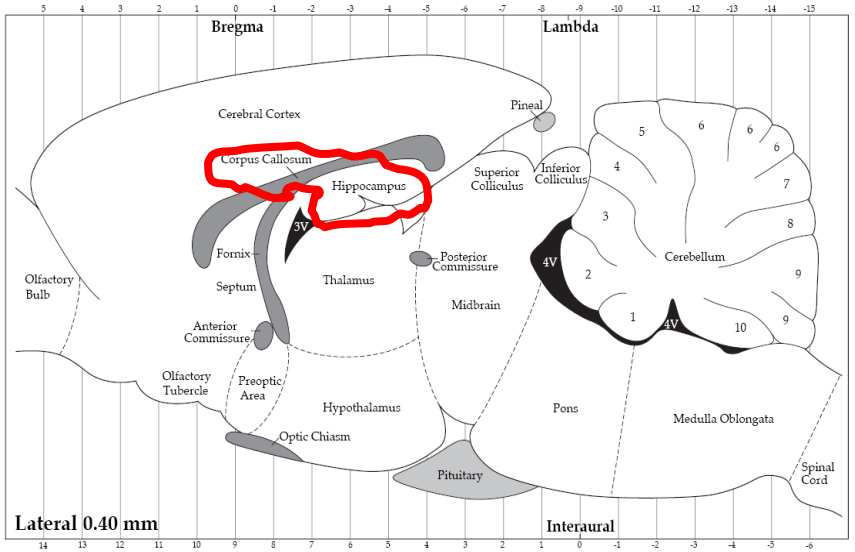
- La funzionalizzazione completa delle NP consente la loro localizzazione a livello tumorale.
- La localizzazione delle NP nell'area di danno uccide le cellule tumorali e riduce la massa.
- La ridotta crescita tumorale si traduce in una migliore prognosi.

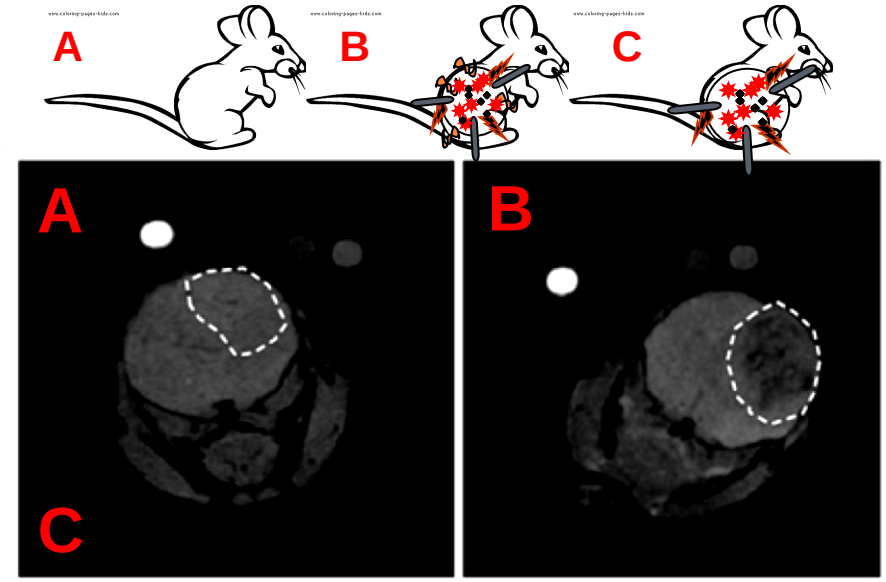
Obiettivi complementari (outcomes secondari)!

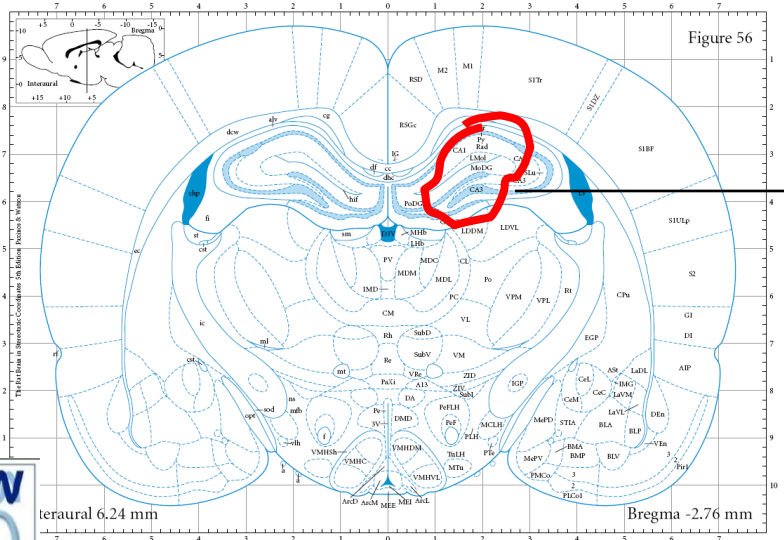
- La funzionalizzazione con agenti di contrasto permette di visualizzare le cellule.
- Le NP penetrano efficacemente all'interno delle cellule bersaglio.
- Il meccanismo di morte cellulare (apoptosi) corrisponde a quello atteso.

1. SOLO LE NPs CON TOTALE FUNZIONALIZZAZIONE LOCALIZZANO NELLA SEDE DELLA CRESCITA DEL TUMORE

MRI con GADOLINIO





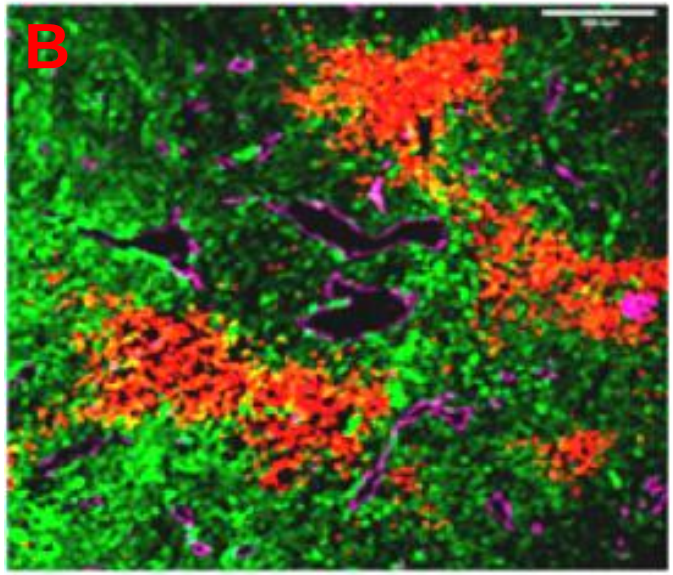


Microscopia

SACRIFICIO

Rodamina NP (rosso)

U87-MG
Verdi fluorescenti



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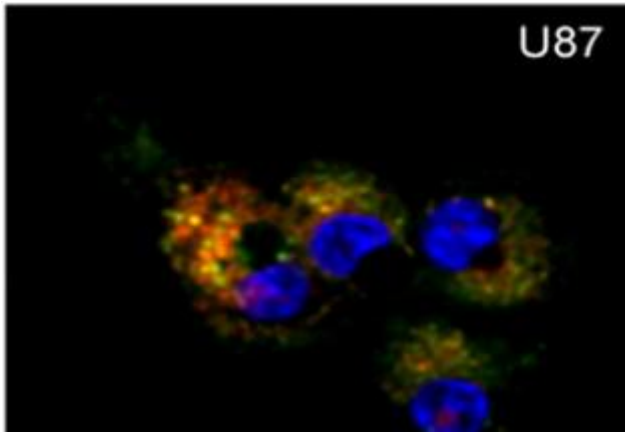
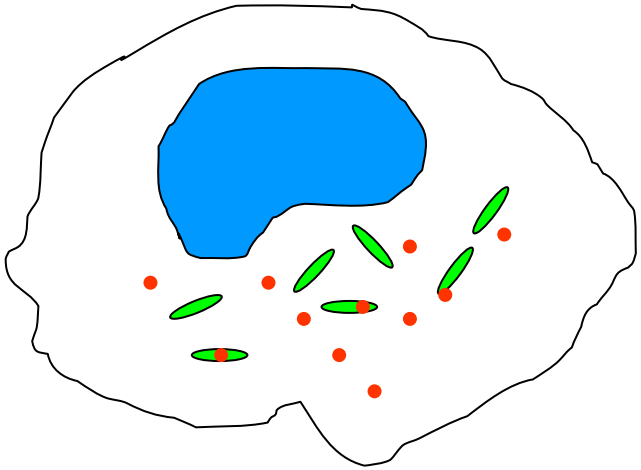
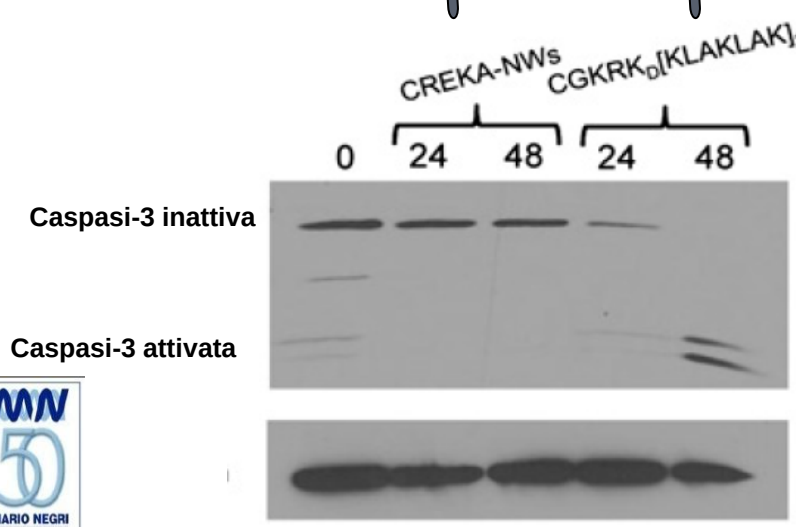
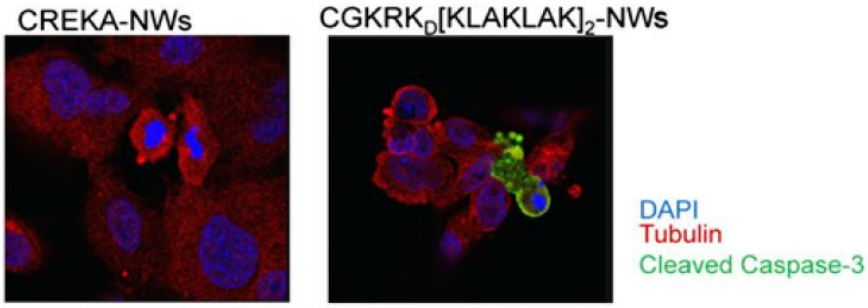
2. LE NPs PENETRANO NELLE U87-MG……



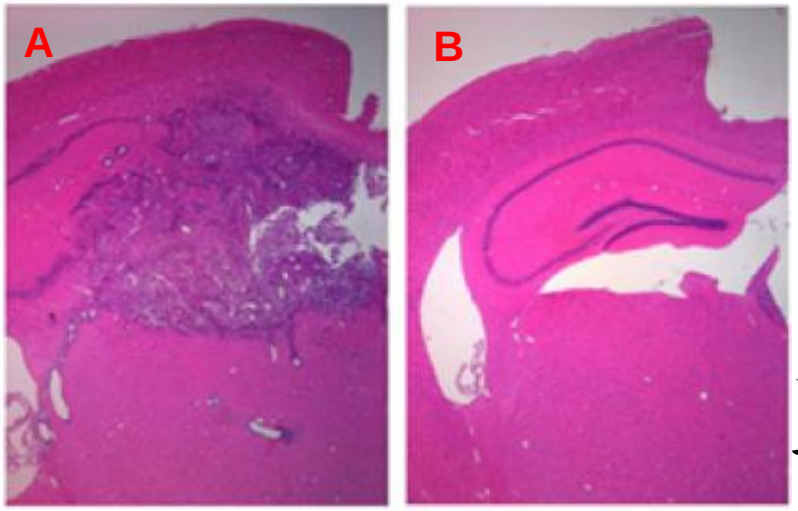


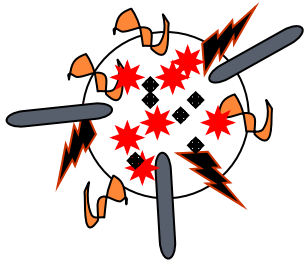
MA SOLO QUELLE PRESENTANTI IL PEPTIDE CITOTOSSICO ATTIVA MECCANISMI DI MORTE PROGRAMMATA

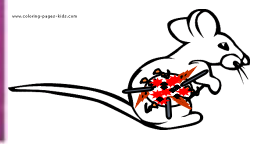




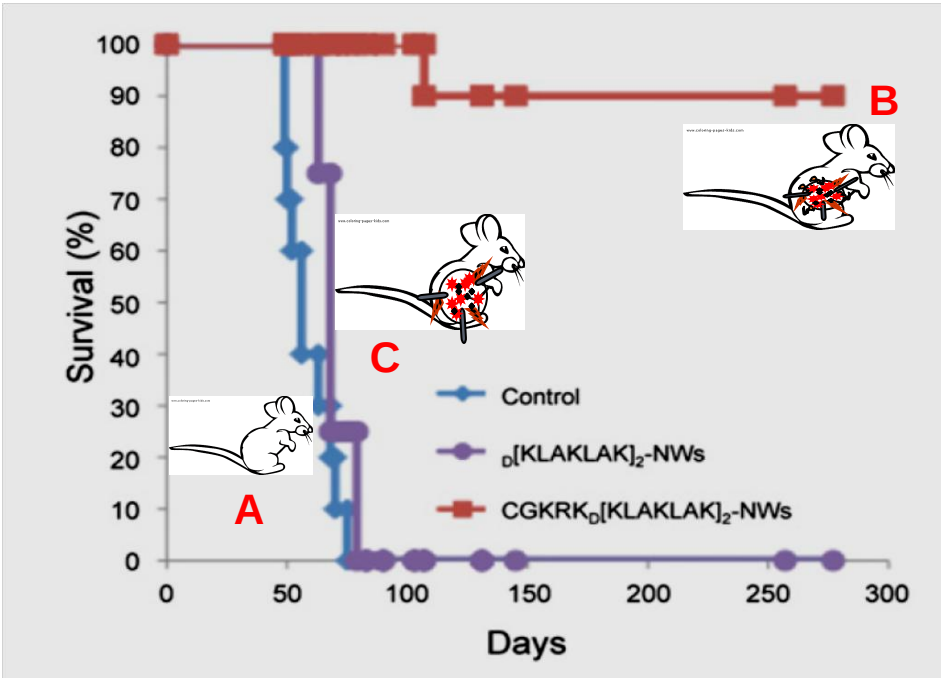
3. IL TRATTAMENTO CON LE NPs ALTAMENTE FUNZIONALIZZAZIONE ARRESTA LA CRESCITA DEL TUMORE….





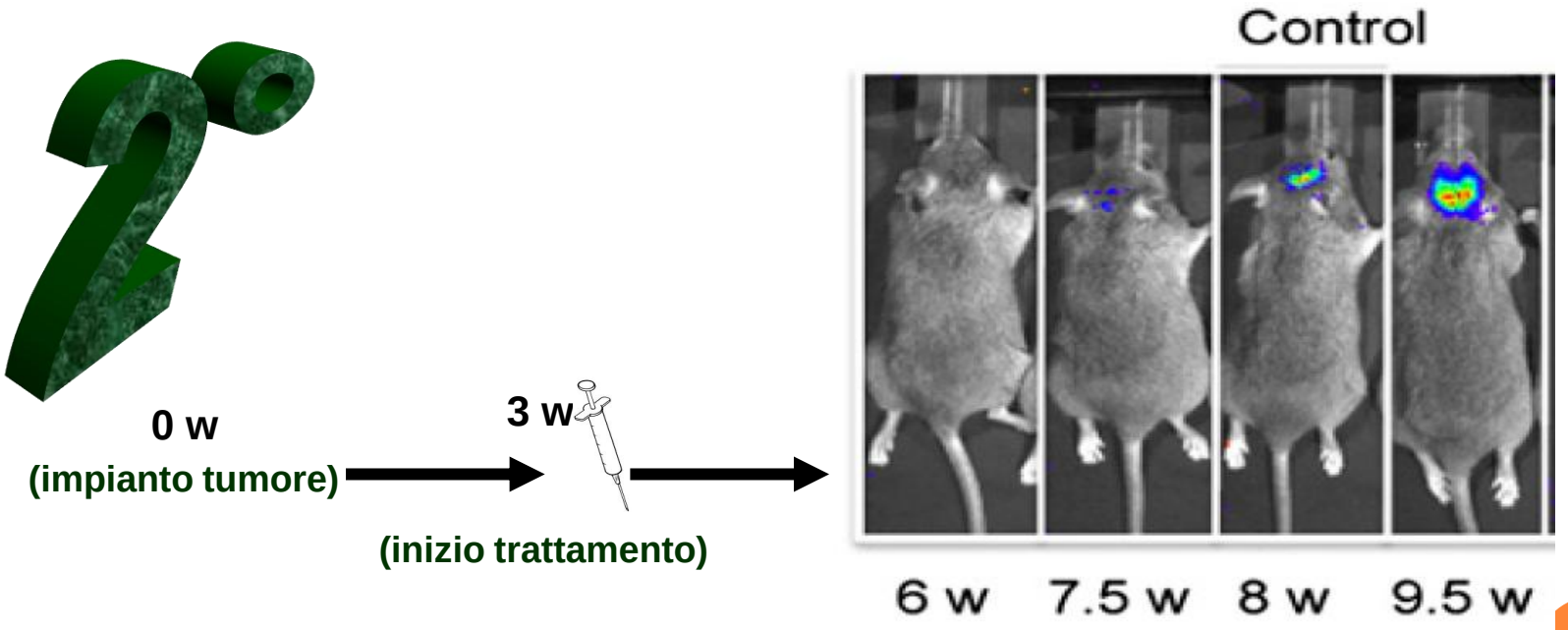


E AUMENTA LA SOPRAVVIVENZA DEL GRUPPO “B”



STUDIO POTENZIALMENTE MOLTO INTERESSANTE MA……

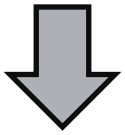
1. MANCA IL GRUPPO RICEVENTE IL FARMACO LIBERO ( )



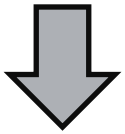
TUMORE POCO AGGRESSIVO O TRATTAMENTO PRECOCE

COME SVILUPPARE UN NANO-FARMACO?

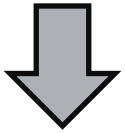
FASE 1: SINTESI NANO-STRUTTURA
(ING. CHIMICI)



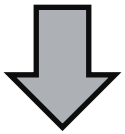
FASE 2: FUNZIONALIZZAZIONE NANO-STRUTTURA
(CHIMICI FARMACEUTICI)



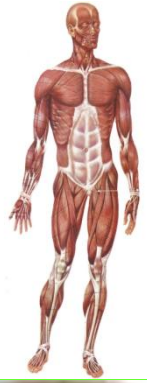
FASE 3: INTERAZIONE NANO-STRUTTURA CELLULA
(BIOLOGI CELLULARI)



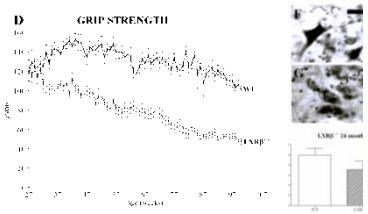
FASE 4: INTERAZIONE NANO-STRUTTURA ORGANISMO
(FARMACOLOGI)



FASE 5: EFFETTO TERAPEUTICO/EFFETTO TOSSICO
(BIOLOGI, VETERINARI, MEDICI)



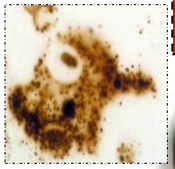
EFFICIENCY



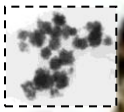
TRACKING

MICE

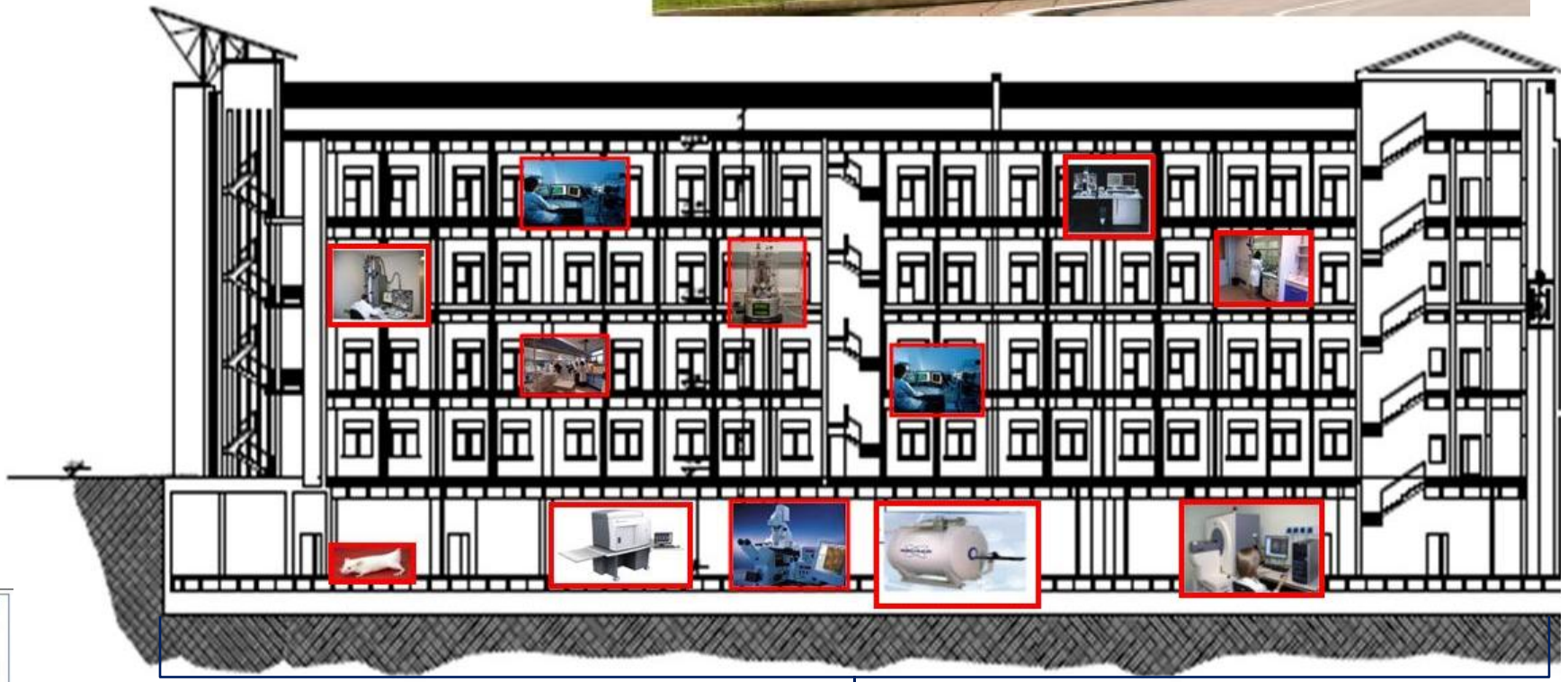
CELLS



NPS



UN ESEMPIO CONCRETO……



2 PROGETTI IMPORTANTI

Molecular basis for triple negative breast cancer metastasis: new tools for diagnosis and therapy

Nanoparticles for the therapy and diagnosis of Alzheimer Disease

AIRC

SEVENTH FRAMEWORK PROGRAMME

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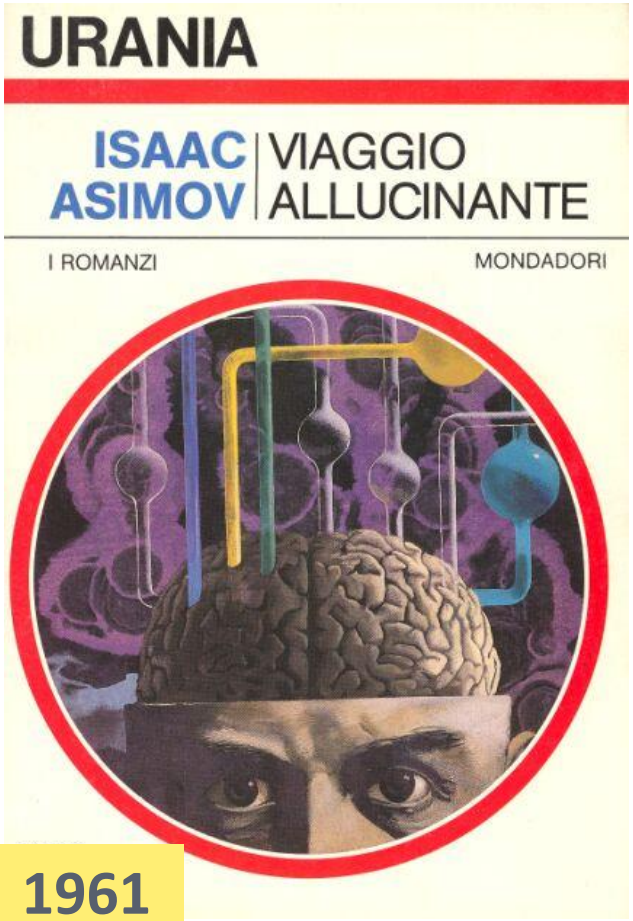
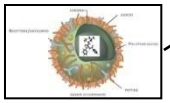
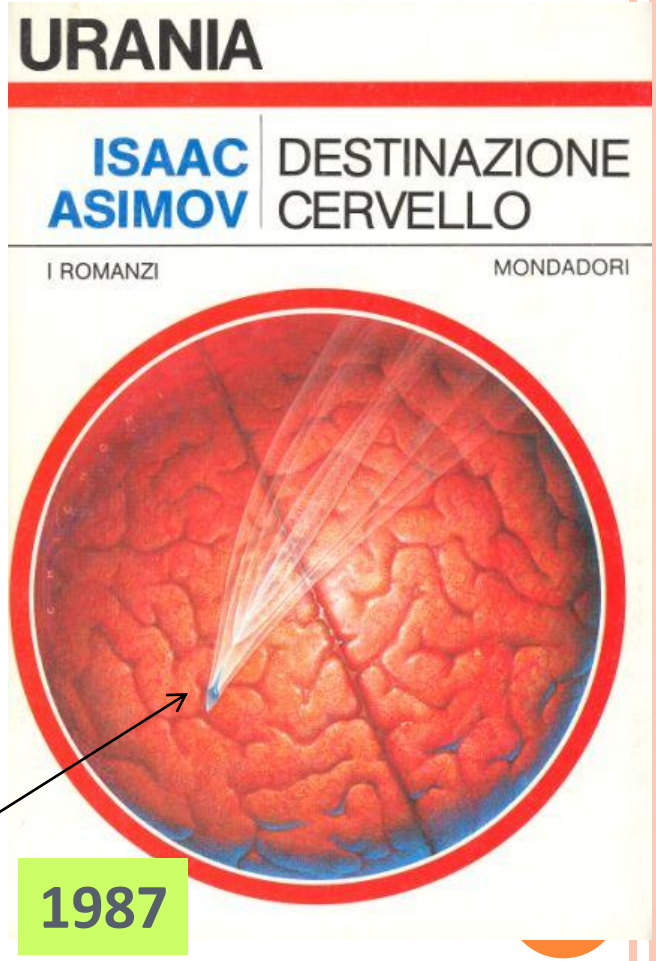
DALLA FANTASCIENZA



1961





1987

ALLA SCIENZA

"Possiamo immaginarci un futuro in cui nanostrutture piccoli ed intelligenti possano correre libere nel sangue, raggiungere bersagli patologici specifici, interagire con essi e rilasciare farmaci per riparare tessuti, distruggere cellule impazzite o annientare invasori estranei"

GRAZIE !!!!

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ISTITUTO DI RICERCHE FARMACOLOGICHE
1961 - 2011