Programa Cooperación Farma-Biotech

Jornada IIb: **Oncología**

EDAHPVE7 as a therapeutic vaccine against cervix carcinoma

DIGNA BIOTECH

Madrid, 12 de mayo de 2011

CataloniaBIO
Associació catalana d'empreses de biotecnologia

MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española

GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN

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1.The Company

DIGNA BIOTECH is the forefront of a large-scale pioneering biopharmaceutical initiative in Spain

University of Navarre

IP portfolio & Expertise

Traslacional research: oncology, cardiology, CNS, hepatology

$$$

Consortium of 15 Investors with 150M€ Commitment 2002-2012

Tech transfer

$$$

$$$

BiTA

IP management

IP transfer

DIGNA BIOTECH

Development and licensing

Intense collaboration with U. Navarra research support network, and companies with common investment base (manufacturers 3P and DRO, regulatory expert Idifarma)

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DIGNA BIOTECH evolution 2004 – 2010
Position at 31st December 2010

2005:	**7 patents**	**4 products**	**4 persons**	**Capital: 1,7M**
2010:	**45 patents**	**33 products**	**20 persons**	**Capital: 16,2M**

Co-development income: **11,4M**

Credit +Grants: **15,0M**

Direct investment:	27,0M
Operative costs:	10,5M
Invesment/Costs:	**73% / 27%**
Investment engaged for licences:	20,5M

Identified projects:	Licensed products:	Patented products:
42	6	36

Products in clinical phase in 2011: 3

Phase IIb	P144 cream in systemic sclerosis
Phase II	CT-1 in renal transplant
Phase I/II	Interferon alfa 5 in Hepatitis C
Phase I	CT-1 in healthy volunteers

Product reaching clinical phase in 2012: 4

Phase II	CT-1 Hepatectomy
Phase I	AAV vector for porphyria

Products in pre-clinical phase PoC in animal model: 14

Products idenfied in I+D: 24

2. The Product

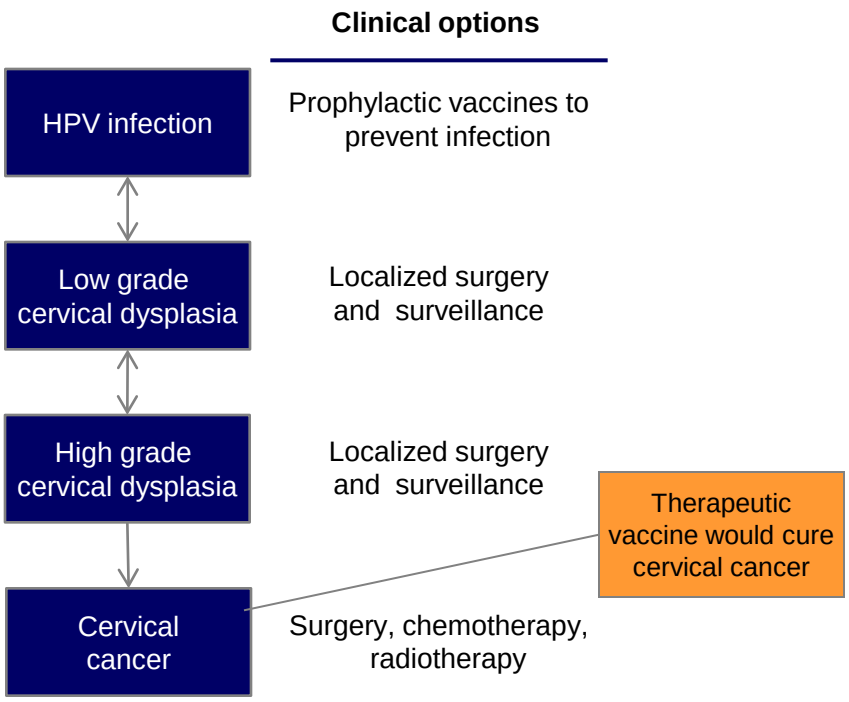
a) Therapeutic focus
b) Innovative mechanisms of action
c) Differential features facing the market
d) Current status of development
e) IPR protection
f) Pitfalls & Risks to be considered

Therapeutic focus: Cervical cancer therapeutic vaccine

HPV affects a majority of young women

- **Human Papillomavirus (HPV) is responsible for ~500.000 cervical cancer cases each year**
  - There are 275.000 associated deaths worldwide each year
- **50-80% of sexually active women are infected at least once in their life**
  - Women are usually infected in their 20s and early 30s
  - Cervical cancer occurs most commonly among women in their 40s and 50s
- **Six types of HPV account for about 85% of cervical cancer cases worldwide**
  - Two HPV types, 16 and 18, account for around 70% of cases
- **Currently there are two prophylactic vaccines against HPV, consequently a reduction in the incidence rate of the infection is expected**

HPV infection originates cervical dysplasia, which can result in cancer

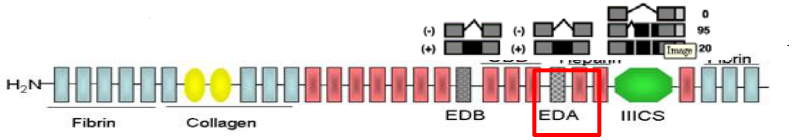


Human papillomavirus (HPV) infection is the major risk factor for the development of cervical cancer

Sources: HPV and Cervical Cancer: Unique challenges and opportunities for disease prevention. July 2005, Path .org;. *Epidemiologic natural history and clinical management of Human Papillomavirus (HPV) Disease: a critical and systematic review of the literature in the development of an HPV dynamic transmission model.* BMC Infectious Diseases 2009, 9:119

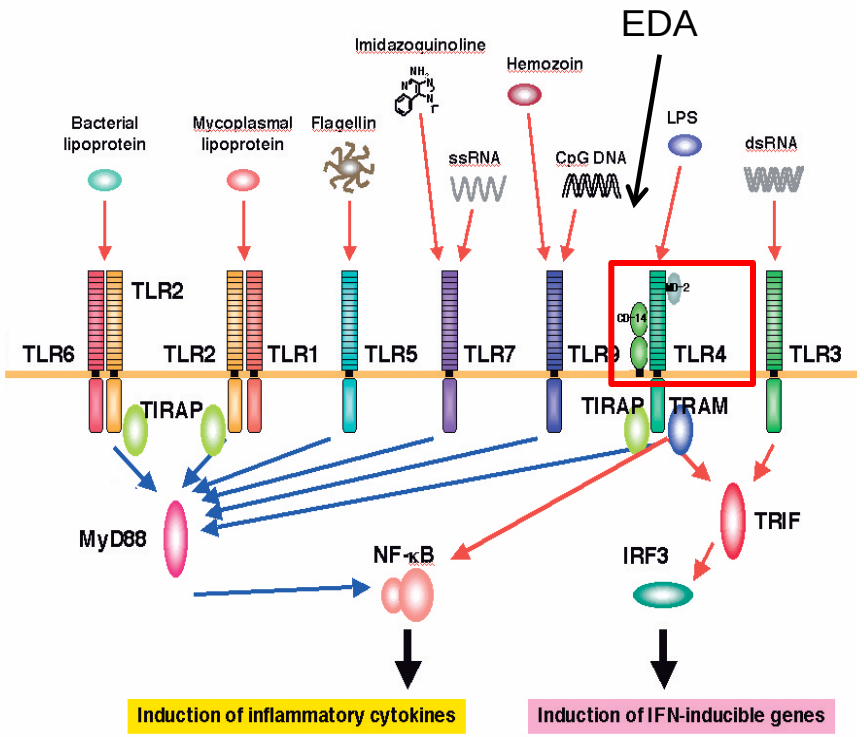
Innovative mechanisms of action: EDA signalling pathway

Fibronectin Extra Domain A (EDA)

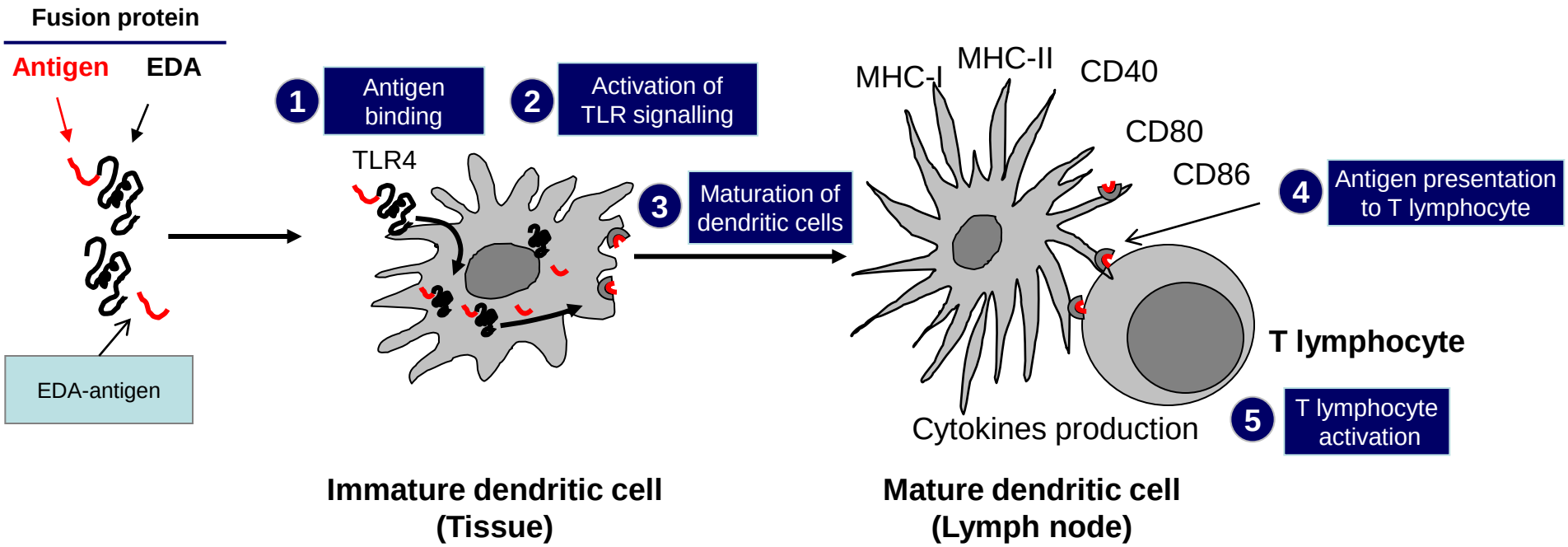


- Fibronectin is a multidomain glycoprotein involved in several cellular processes, including tissue repair, embryogenesis, blood clotting and cell migration/adhesion
- Extra Domain A from fibronectin (EDA) is produced by alternative splicing of fibronectin in response to tissue injury (Rheumatoid arthritis, wound healing, epithelial fibrosis, vascular intimal proliferation, inflammation).
- EDA is related to the immune system:
  - Induces expression of proinflammatory citokines
  - Activates TLR4 signaling.

EDA signaling pathway



Innovative mechanisms of action: Rationale



Activation of dendritic cells is a key step in the immune response, because they are key regulators of T and B lymphocytes

Source: The extra domain A from Fibronectin targets antigens to TLR4-expressing cells and induces cytotoxic T cell responses in vivo, J Immunol, 2007; 178: 748-756

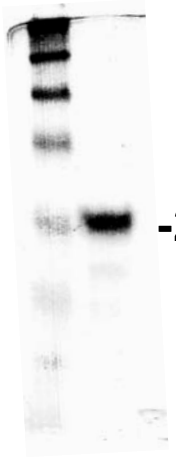
Innovative mechanisms of action: Construction of EDA fused to Human papilomavirus E7 protein, EDAHPVE7

A

B

MHGDTPTLHEYMLDLQPETTDLYCYEQLNHMNIDR
PKGLAFTDVDVDSIKIAWESPQGQVSRYRVTYSSPE
DGIRELFPAPDGEDDTAELQGLRPGSEYTVSVVAL
HDDMESQPLIGIQSTAAAGQAEPDRAHYNIVTFCCK
CDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQK
PAAALEHHHHHH

C



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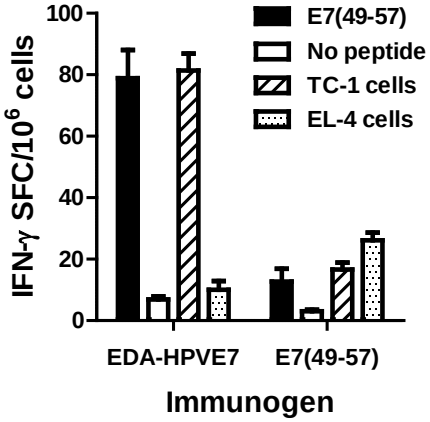
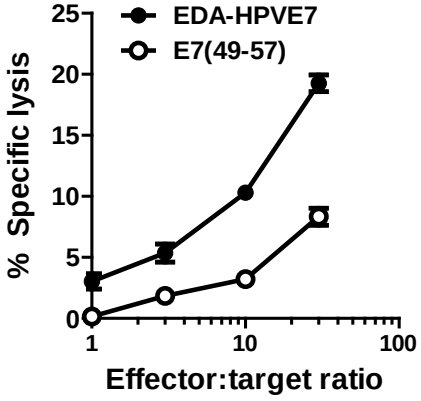
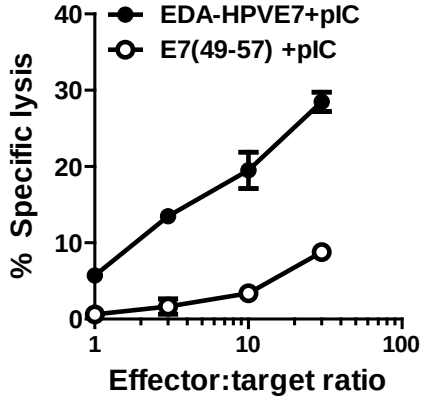
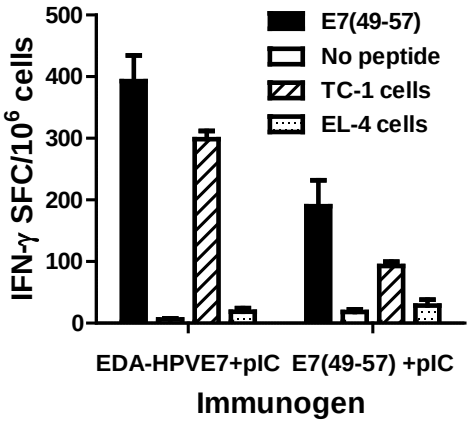
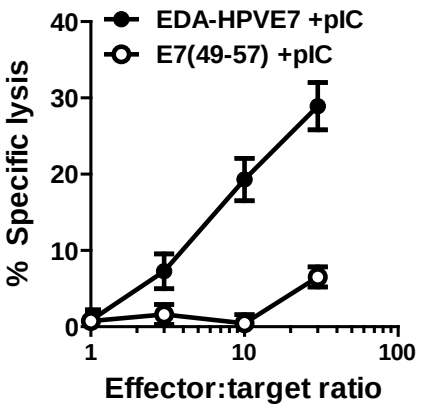
Innovative mechanisms of action: Cellular immune response induced against HPVE7 after immunization with EDAHPVE7





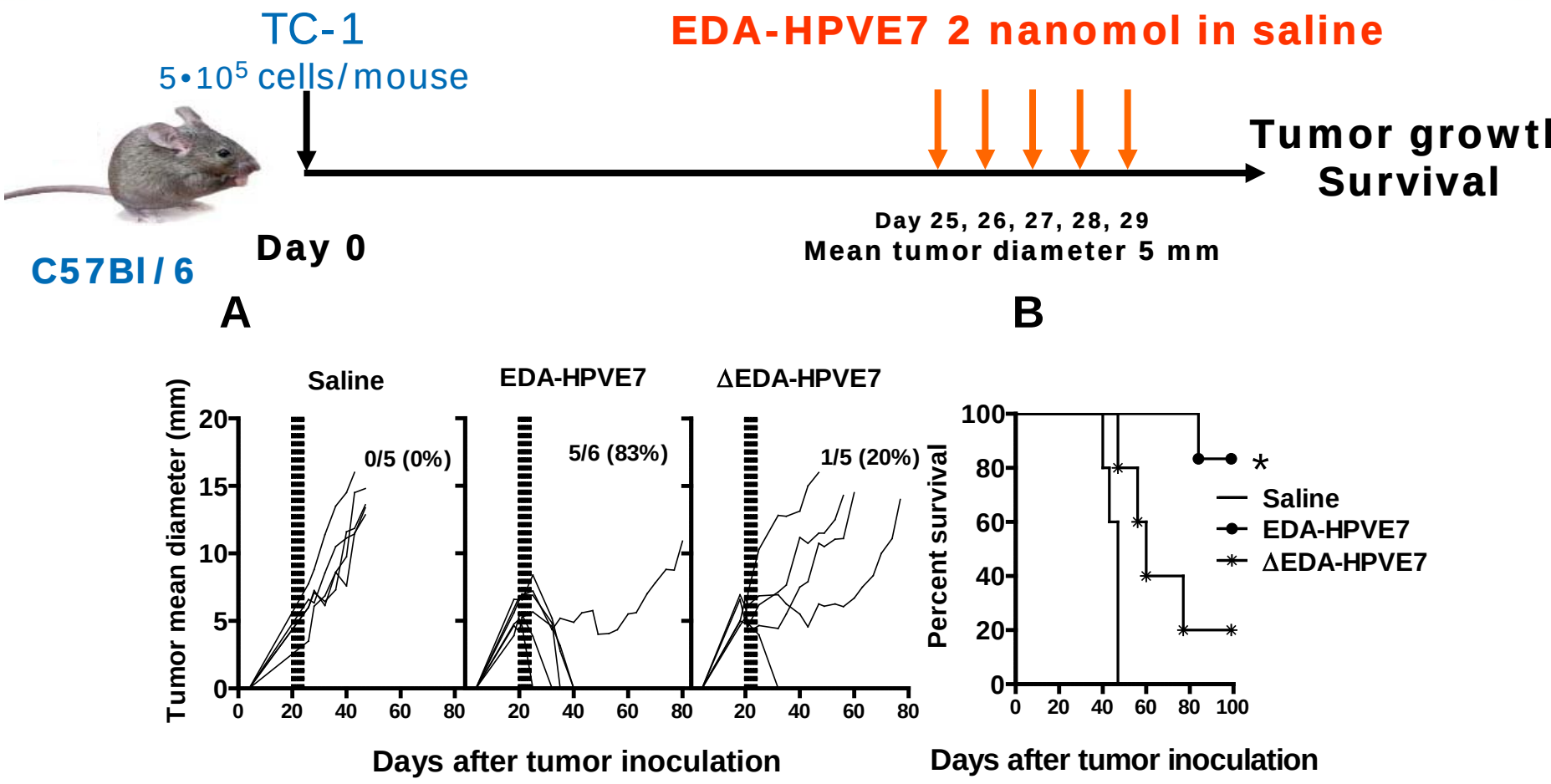






***In vivo* induction of cellular immune responses against HPVE7 by immunization with EDA-HPVE7 protein**. Mice were immunized i.v. with 2 nmol of EDA-HPVE7 or with peptide E7(49-57) with or without PIC (B and A respectively). Seven days after immunization, mice were sacrificed and spleen cells were cultured in the presence or absence of E7(49-57). After 5 days of culture, CTL activity against peptide E7(49-57) target cell pulsed with the same peptide was measured.

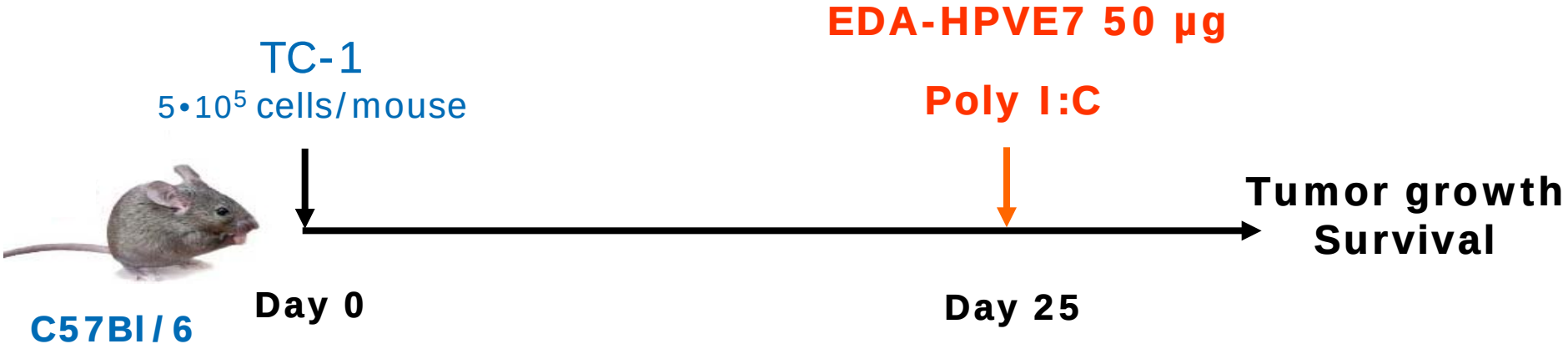
Innovative mechanisms of action: Therapeutic effect of repeated administration of EDA-HPVE7 in the absence of adjuvants on TC-1 tumor bearing mice

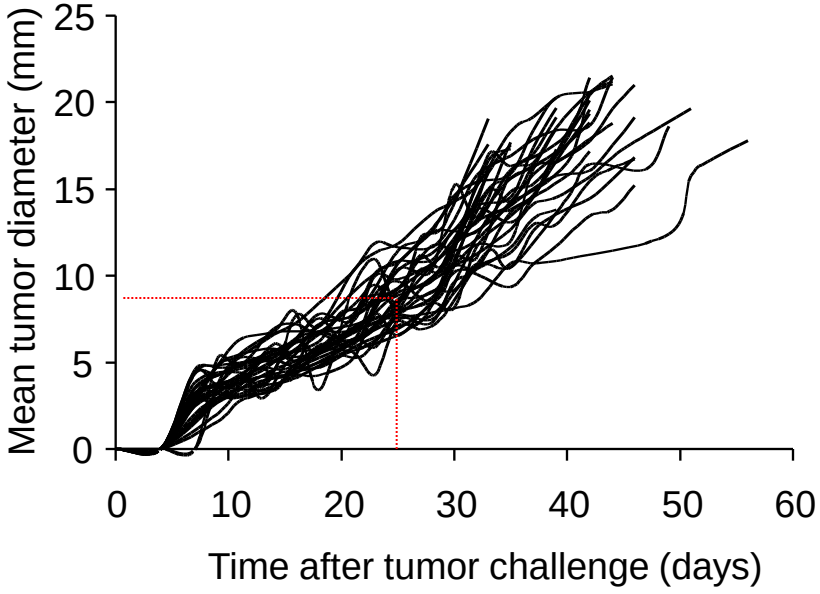


Therapeutic Efficacy of EDA-HPVE7 fusion protein in saline. C57BL/6 mice were injected s.c. with 5 x 105 TC-1 cells and 20 days later, when tumor mean diameters were around 5-7 mm, mice were treated i.t during 5 consecutive days with EDA-HPVE7 or with ΔEDA-HPVE7 (containing a truncated version of EDA).

Mansilla et al In J Cancer, 2011 Under review

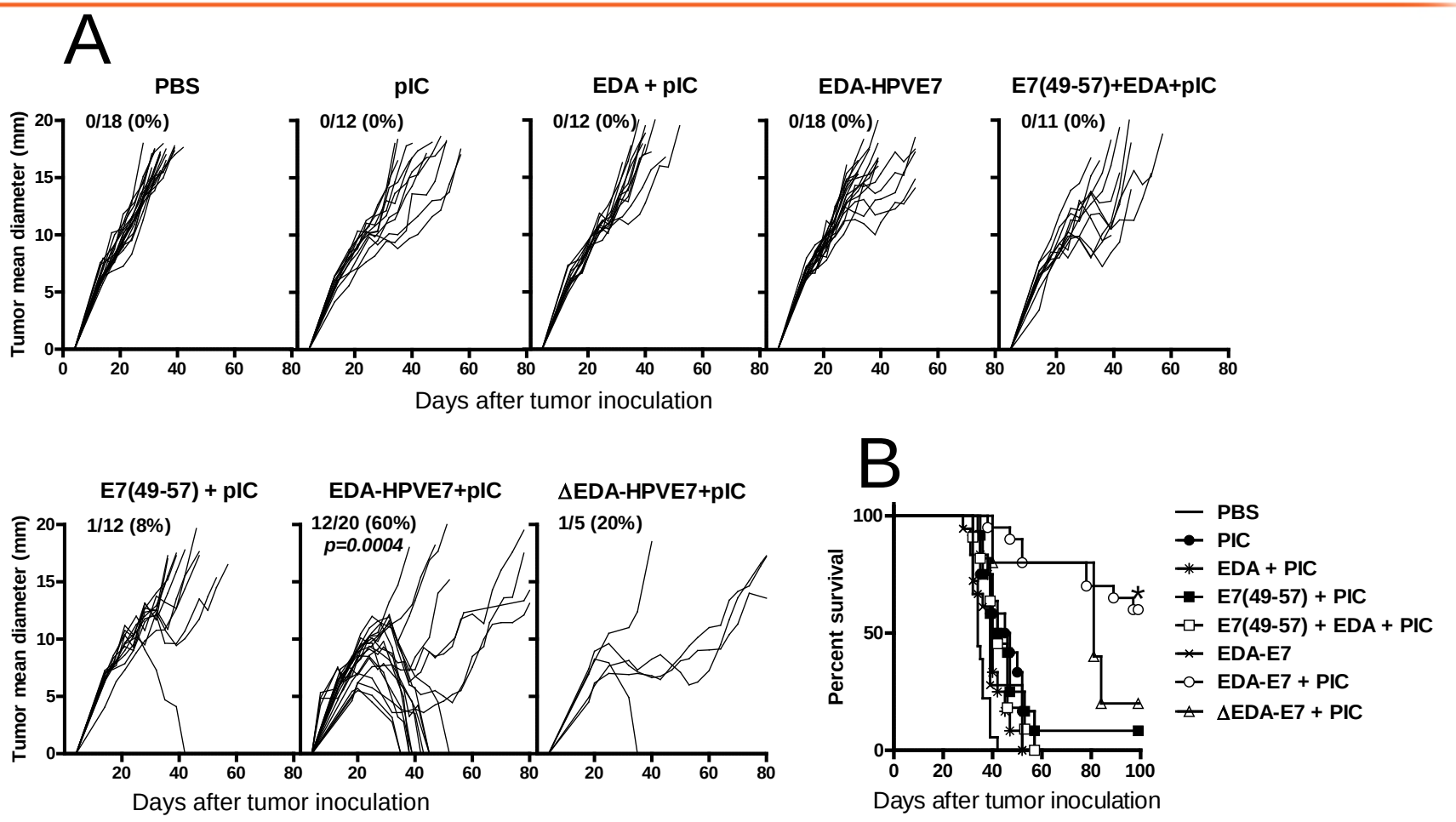
Innovative mechanisms of action: Treatment of TC-1 established tumors: Day 25





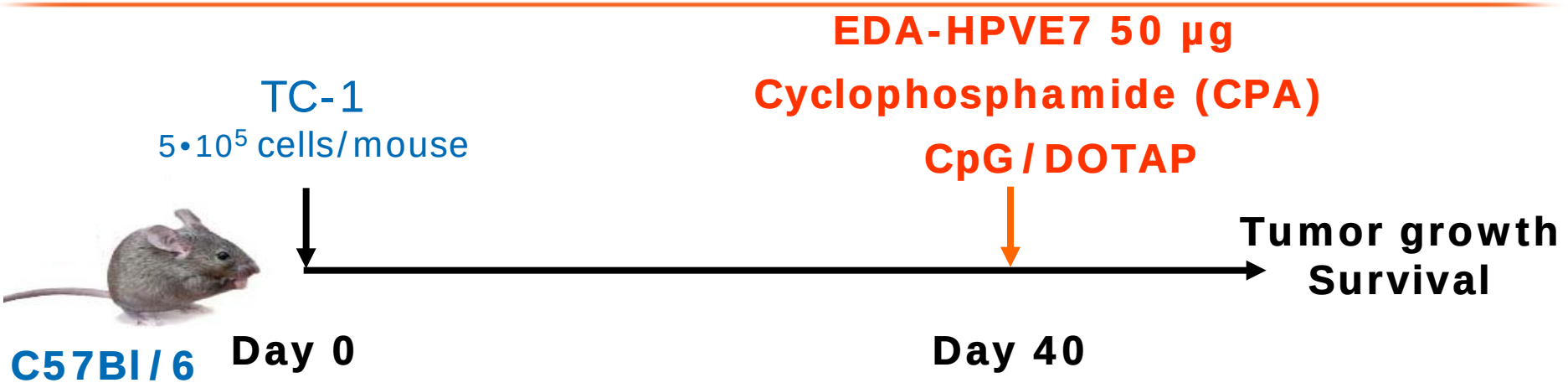
Day 25 - 9 mm

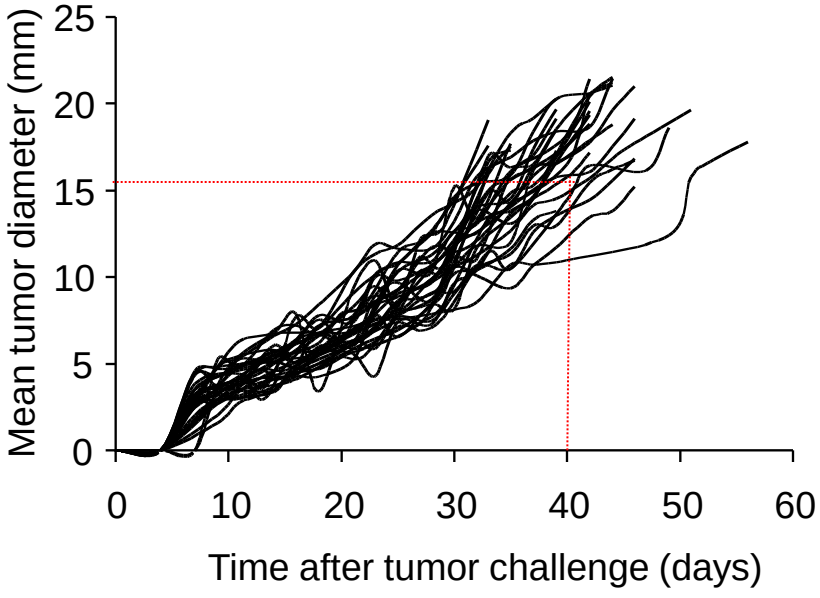
Innovative mechanisms of action: Therapeutic efficacy of EDA-HPVE7 fusion protein combined with poly I:C



Therapeutic Efficacy of EDA-HPVE7 fusion protein combined with pIC. C57BL/6 mice were injected s.c. with 5 x 10^5 TC-1 cells and 25 days later, when tumor mean diameters were around 8 mm, mice were treated intravenously with different combinations of EDA vaccine.

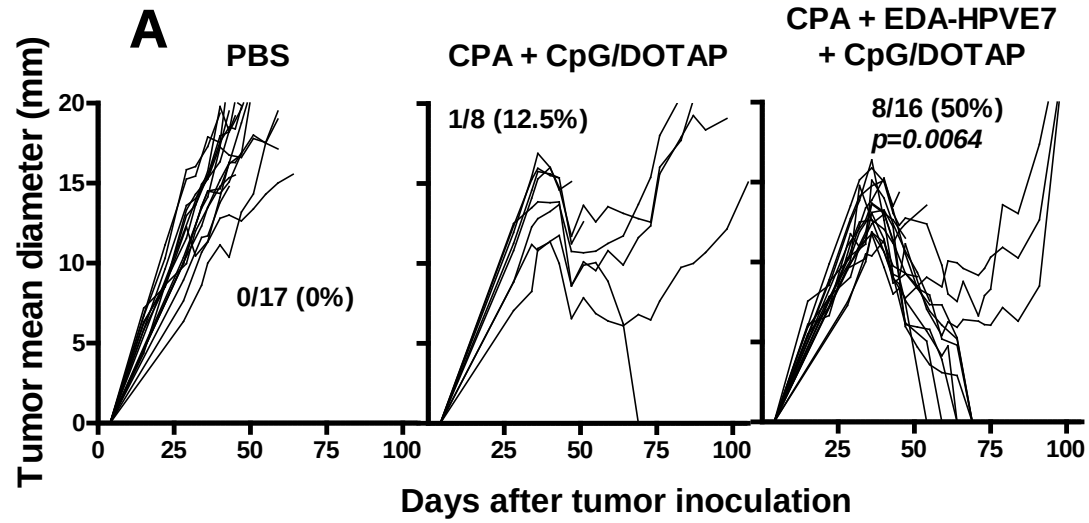
Innovative mechanisms of action: Treatment of established TC-1 tumors: Day 40

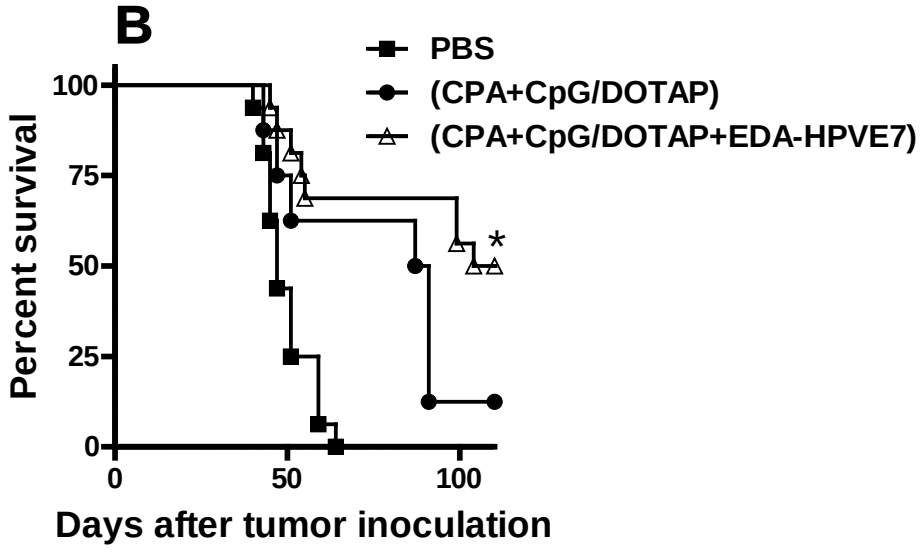




Day 40 - 16.5 mm

Innovative mechanisms of action: Treatment of established TC-1 tumors: Day 40





Therapeutic efficacy of EDA-HPVE7 fusion protein in a model of large TC-1 tumors. C57BL/6 mice were injected s.c. with 5 x 10^5 TC-1 cells On day 36, when tumor mean diameters were around 12 mm, and on day 51, mice were injected with PBS or 30 mg CpG-B/DOTAP i.v. or with 50 mg EDA-HPVE7 plus 30 mg CpG-B/DOTAP i.v. Mice were treated with saline or with 175 mg/kg cyclophosphamide (CPA) i.p. at days 35 and 50 (one day before each immunization). (B). (*;p<0.05 when compared mice treated with tritherapy vs. mice treated with CPA+ CpG-B/DOTAP).

Mansilla et al In J Cancer, 2011 Under review

Differential features facing the market: Advantages of EDA-Fusion Protein Vaccines

- Efficient Antigen Delivery To And Simultaneous Maturation Of Dendritic Cells
- Robust Th1 Cell And Cytotoxic T Cell Responses
  - Necessary for Tumor Destruction and Elimination of Persistent Viral Infections
- Low Humoral Immune Response To EDA Moiety After Vaccination
  - No Obstacle To Repeat Immunizations
- Synergy With Other Vaccine Platforms and/or TLR 7 agonist
- Human origen of the protein
- EDA fusion proteins as a Platform for generates different candidates already proven with EDA-NS3; EDA-MLM

Differential features facing the market: EDA offers several advantages over alternative TLR-adjuvants

TLR dependent adjuvants family	Description	Origin	Fusion protein	Single adjuvant	Main application	Receptor location	Myeloid / Plasmacytoid response
TLR-9 ligand (DNA-based)	• Unmethylated CpG dinucleotides from bacteria or virus	Bacterial Viral	✓	✓	Therapeutic / Prophylactic	Endosome	Plasmacytoid
TLR-5 ligand (Flagellin)	• Protein from the bacterial flagellae	Bacterial	✓	✓	Prophylactic	Cellular membrane	Myeloid
TLR-4 ligand (MPL-derived)	• Derived from detoxified lipopolysaccharide from *Sal. minnesota*	Bacterial Synthetic	—	—	Prophylactic	Cellular membrane	Myeloid
TLR-4 ligand (EDA)	• Derived from the human protein fibronectin	Human	✓	✓	Therapeutic (Prophylactic)	Cellular membrane	Myeloid

EDA advantages

- Human origin induces less reactogenicity against adjuvant
- Fusion protein promotes antigen binding and ensures coupling of antigen delivery and adjuvant action
- Single adjuvants are typically simpler from a development and regulatory viewpoint
- Therapeutic vaccines are usually focused on more attractive markets
- Cellular membrane location more accessible to target than internal endosome
- Myeloid dendritic cells generate more effective immune responses

Source: Medtrack database. Stakeholder opinions: Vaccine adjuvants. Datamonitor, September 2008; Stakeholder opinions: Therapeutic vaccines. Datamonitor, December 2009; Biolty analysis

Differential features facing the market: Therapeutic HPV vaccine advantages and disadvantages

Therapeutic HPV vaccine advantages and disadvantages and future strategies

	Advantages	Disadvantages	Future Prospects
Live Vector-based	Numerous vectors available. Highly immunogenic. Can be engineered to express cytokines and co-stimulatory molecules.	Pre-existing immunity. Possible dominance of immune response to viral vector rather than HPV antigen. Neutralizing antibodies restrict repeated administration. Risk of disease.	Enhancement of immunogenicity through adjuvant and fusion proteins. Circumvention of neutralizing antibodies to allow repeat dosage.
Peptide- based	Safe. Stable. Easy to produce. Can combine multiple epitopes in long chain peptides.	Epitopes must be determined. HLA-restriction. Low immunogenicity.	Enhancement of immunogenicity through. Epitope enhancement. Lipopeptide delivery.
Protein-based	Safe. Stable. No MHC restriction.	Low immunogenicity; limited CTL response.	Enhancement of immunogenicity through adjuvant and fusion proteins.
DC-based	Highly immunogenic. Multiple methods available to load antigen.	Expensive. Labor-intensive as individualized. Lack of agreed standards for preparation. DCs do not necessarily home to lymph nodes.	Increase survival of DCs. More efficient loading of antigen. Identification of the most effective delivery route.
Tumor-cell based	Likely to express tumor antigens.	Safety concerns. Labor-intensive as individualized. Weak antigen presentation by tumor cells.	Address safety issues. Immunogenicity enhanced by cytokines. Consistency in potency and purity established.
DNA-based	Safe. Stable. Easy to produce. Can administer multiple times. Several delivery methods possible. More sustained expression of antigen.	Low immunogenicity. No intercellular spreading. Risk of genomic integration.	Increase number and lifespan of antigen-expressing DCs. Enhanced DC antigen processing and presentation. Improve DC interaction with T cells.
RNA-based	Safe. Transient, non-infectious. Can administer multiple times. No risk of genomic integration.	Difficult to produce and store - unstable. Labor intensive to produce. No intercellular spreading.	Improved DNA-launched RNA replicons. Prevention of early apoptosis.

From Lin et al. J.Formos Med Assoc.2010 January

Differential features facing the market: Companies developing therapeutic cervical cancer vaccines

Current cervical cancer treatment includes surgery, radiotherapy and chemotherapy

- **Surgical procedures remove the cancer**
  - There are several types of surgeries which are able to remove the uterus, cervix, or other near organs which might be affected
  - Used mostly in early and medium stages
- **Radiation therapy uses high-energy x-rays or other types of radiation to kill cancer cells**
  - The radiation can be administered through external devices or internally (using a radioactive substance placed directly near the cancer)
  - Used mostly in medium and late stage
- **Chemotherapy makes use of different drugs to eradicate cancer**
  - Common drugs include: carboplatin, cisplatin, paclitaxel, fluororacil, cyclophosphamide
  - Usually a combination of drugs is administered
  - Often given concurrently with radiotherapy

Vaccines play a major role in the cervical cancer development pipeline

	Company	Product	Target	Phase
Small molecules	Bristol-Myers Squibb	Erbitux[1]+Cisplatin + Radiotherapy	EGFR, DNA	II
	Lilly	Alimta	Folate-dep. enzymes	II
	GlaxoSmithKline	Votrient + Tykerb	VEGF;c-Kit; PDGF-R	II
	NEKTAR	PEG irinotecan	Topoisomerase	II
	NOVOGEN	Phenoxodiol	XIAP, FLIP inhibitor	I+D
Vaccines	ADVAXIS	ADX11001	HPV16 E7	I
	ISA Pharmaceuticals	ISAHPV01	HPV16 E6/7	I
	APOIMMUNE	ApoVax104	HPV16 E7	PC
	PDS Biotechnology	PDS0101	HPV16 E7	PC

Note: 1 Erbitux is a monoclonal antibody, not a small molecule
Source: American cancer society website; Cancer research UK website; National Cancer Institute website; Medtrack database, Companies websites

Differential features facing the market: Companies developing therapeutic cervical cancer vaccines

Cervical carcinoma vaccines pipeline

	Company	Product	Molecule	Phase	Characteristics versus EDA
No adjuvant	ISA Pharmaceuticals	ISAHPV01	Synthetic Long Peptide containing nine overlapping E6 and E7 peptides	I	- Not adding an adjuvant limits intensity or persistence of immune response, potentially limiting efficacy
Ag-encapsulated	PDS Biotechnology	PDS0101	Lipidic nanoparticles with HPV16 E7 antigen	PC	- Non human origin could induce immunotoxicity against adjuvant - Delivery vehicle, no immune system enhancer - Delivery not very specific, limiting efficacy
Immuno stimulant	APOIMMUNE	ApoVax104	Streptavidin + 4-1BB ligand and HPV16 E7 antigen	PC	- EDA elicits a more comprehensive immune response, activating several related immune system pathways and acting at different levels in the immune response - Usage mainly as mixture has the risk of decoupling antigen delivery from adjuvant action, reducing efficacy
EDA (TLR-4)	DIGNA BIOTECH	EDA cervical cancer vaccine	EDA+ HPV16 E7	PC	- Human origin reduces immunotoxicity against adjuvant -Fusion protein ensures antigen delivery and binding -Receptor located in myeloid dendritic cells, generating a more effective immune response
Vector	ADVAXIS	ADX11001	*Listeria monocytogenes* secreting HPV16 E7	I	- Non human origin could induce immunotoxicity against vehicle - Risk of development of replication-competent virus - Potentially additional side effects due to genetic mechanism - Risk of contamination

Sources: Companies websites, Clinicaltrials,com, May 2010; Biolty analysis

Current status of development:

-**Positive PoC in the most accepted model of the disease.**

-**Positive PoC in established tumors in combined treatment with other TLR agonist (TLR 7)**

-**Starting toxicological studies:**

- Acute toxicology
- Local tolerance
- Immunotoxicity

-Full develpoment plan till Phase I in place

Current status of development: Fusion proteins produced at CIMA

Product	Indication	Status
EDA-HPVE7	Cervical carcinoma	Preclinical
EDA-MLM	Melanoma	Preclínical
EDA-gp 120	HIV	Discovery
EDA-p24	HIV	Discovery
EDA-HBsAg	Hepatitis B	Discovery
EDA-Mesothelin	Panchreatic cancer	Discovery

IPR protection: EDA current IP includes one approved patent covering the EDA platform and two filed patents focused on particular indications

Agents and methods based on the use of EDA domain of fibronectin ES 200501412	Therapeutic compositions for the treatment of diseases caused by HPV ES 200901847	New compositions based in the EDA for the treatment of melanoma EP 10382036.1
Phase: Approved in Russia	**Phase:** Filed	**Phase:** Filed
Status: PCT	**Status:** PCT	**Status:** PCT
Filing Date: 13th June 2005	**Filing Date:** 11th September 2009	**Filing Date:** 16th February 2010
Summary: Protect the use of the EDA domain of fibronectin in combination with an antigen, or forming a fusion protein, which can bind to TLR4; the production methods and applications of said agents	**Summary:** Protects a therapeutic compositions for the treatment of diseases caused by HPV and more specifically to compositions comprising at least one antigenic peptide derived from HPV E7 and EDA	**Summary:** Protect methods for the treatment of melanoma by specifically directing an antigen to antigen presenting cells by the use of a conjugate comprising the antigen and a ligand which binds specifically to said antigen-presenting cells

Pitfalls & Risks to be considered:

- GMP protein production for any candidate
- No knowledge on humoral response
- First in class
- Potential of autoinmunity exarcebations
- Small market of the first candidate

3. Availability for cooperation

EDA platform for fusion proteins

Digna will create an spin-off: To mature technology

- IP will be licensed to the new Co for an agreed % of shares + small royalty
- Digna will start development of the candidate under a service contract till the new team is selected and contracted
- New Co will develop 2 candidates

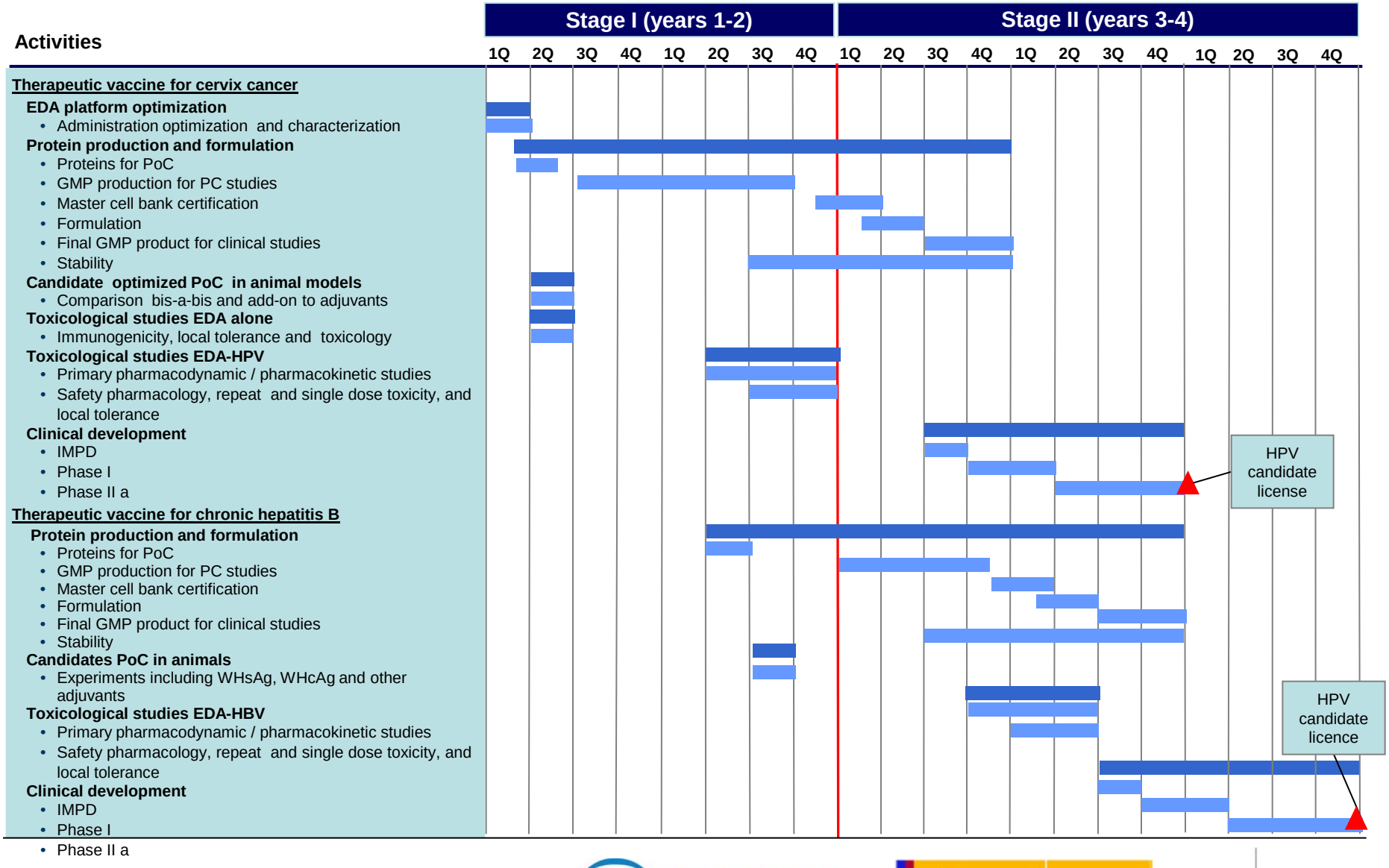
EDA- HPVE7	Therapeutic vaccine for crevical carcinoma
EDA- HbsAg	Therapeutic vaccine for Hepatitis B

EDA Company's plan includes two stages, requiring financing of 3,5 M € over 2 years to complete Stage I and 14,5 M € over 3-5 years for Stage II

	Stage I (year 1-2)	Stage II (year 3-5)
Key objectives	• Substantial advancement in EDA platform optimization and validation • Cervix cancer candidate close to start clinical trials • Chronic hepatitis B candidate with in-vivo PoC	• Cancer cervix candidate with phase IIa completed and in licensing process • Chronic hepatitis B candidate with phase IIa completed and in licensing process • Initial development of additional candidates based on EDA platform
Main activities	• EDA platform optimization • Cervix cancer candidate's comparative PoC in animal models • Cervix cancer candidate's GMP-like production and formulation • Toxicology cervix cancer candidate and EDA alone • Chronic hepatitis B candidate's comparative PoC in animal models	• Cervix cancer candidate's final formulation, GMP production and stability studies • Cervix cancer candidate's clinical development including Phase I and Phase IIa • Hepatitis B candidate's GMP production, formulation, stability • Toxicological studies hepatitis B candidate • Hepatitis B candidate's clinical development including Phase I and Phase IIa
Financial needs (cash)	4,4 M€ 3,5 M€ from private investors (Round 1) 0,9 M€ from grants	14,5M€ 12 M€ from private investors (Round 2) 2,5 M€ from grants

EDA Company's status at the end of Stage I is not expected to offer an interesting exit option for initial investors, due to the early development of its candidates. Additional investment equivalent to Round 2 would be required to achieve additional milestones, offering more attractive exit possibilities.

Operative plan will take first candidate close to clinical stage and start development of second candidate during the first two years



Future targets

Indication	Fusion protein
Hepatitis B	• EDA-WHCAg Woodchuck Hepatitis core antigen fusion protein for POC in animals • EDA-WHS Woodchuck Hepatitis surface antigen fusion protein for POC in animals • EDA-HBsAg human fusion protein for clinical development • EDA-HBcAg human fusion protein for clinical development
Melanoma	• EDA-TRP-2 human fusion protein for clinical development
Pancreatic Cancer	• EDA-Mesothelin human fusion protein for clinical development
Breast Cancer	• EDA- MAGE-B human fusion protein for clinical development
Colon Cancer	• EDA- AH1 (CTDL) GT26 mouse fusion protein for POC in animals
Hepatocarcinoma	• EDA-MuLv mouse fusion protein for PoC in animals
HIV	• EDA-p24 human fusion protein for clinical development • EDAp-120 fusion protein for clinical development