novocure®

TTFields in the Lab

Tools for **Innovation**

TTFields therapy is an innovative cancer treatment developed by Novocure that is currently approved by the United States Food and Drug Administration for the treatment of glioblastoma and malignant pleural mesothelioma and is being investigated in a broad range of other solid tumor types.[1,2,*]

TTFields are alternating electric fields tuned to specific frequencies to disrupt cancer cell division in solid tumors. In investigations of over 30 different cancer cell lines across multiple tumor types, TTFields have consistently demonstrated an anti-mitotic effect *in vitro*.[3,4] Additionally, preclinical research has shown that TTFields delay repair of DNA damage following radiation, reduce cell migration and invasion, and induce autophagy and immunogenic cell death.[5–8]

Novocure is striving to improve survival in some of the most aggressive forms of cancer. To further knowledge about this innovative therapy, Novocure offers several preclinical research tools for use by investigators interested in TTFields.[9,10]

> **Using the inovitro™ system, our team discovered that TTFields application can permeabilize the cancer cell membrane, thereby increasing access of anti-cancer therapies into the cancer cells**
>
> **Chirag Patel**, MD, PhD **and Edwin Chang**, PhD
> Stanford University

> **The expanding topic of TTFields research is very novel and exciting, as it is helping to better elucidate our knowledge of cancer cell plasticity and molecular properties in response to TTFields treatment and how the response differs from traditional pharmacologic or radiation-based therapies**
>
> **Emil Lou**, MD, PhD
> University of Minnesota

*TTFields (200 kHz) are approved in the United States for the treatment of adult patients (22 years of age or older) with histologically confirmed supratentorial GBM by the US FDA through the PMA pathway. TTFields have received a CE mark for marketing authorization in Europe for the treatment of GBM. TTFields (150 kHz) have been approved in the United States by the FDA under the HDE pathway for the treatment of adult patients with unresectable, locally advanced or metastatic, MPM to be used concurrently with pemetrexed and platinum-based chemotherapy.

GBM, glioblastoma; HDE, Humanitarian Device Exemption; MPM, malignant pleural mesothelioma; PMA, premarket approval; TTFields, Tumor Treating Fields.

INOVITRO™
TTFields Cell Culture System

The inovitro™ system enables researchers to study the effects of TTFields *in vitro*.[9]

inovitro System Components

1. TTFields generator
2. Base plate
3. Ceramic dishes
4. Dish covers
5. Mini USB cable
6. Flat cable

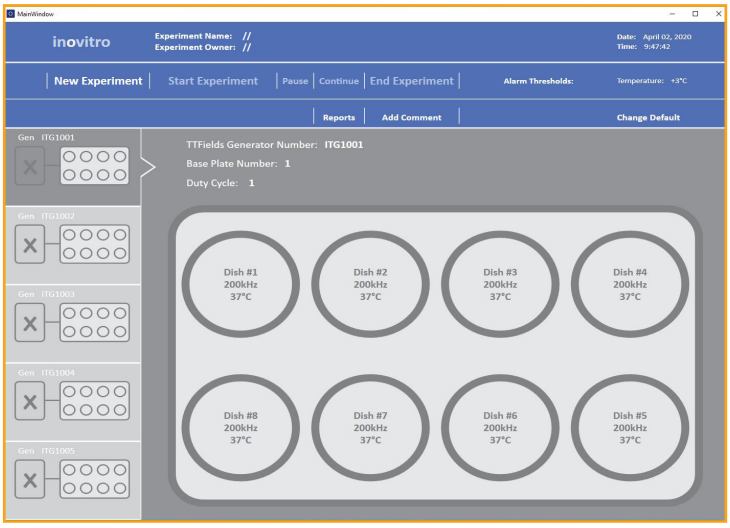
The basic unit of the system is a highly engineered, electrically insulated, **ceramic dish** with arrays embedded that allow the application of TTFields in 2 perpendicular directions at different frequencies and intensities to cell cultures.

The ceramic dishes (8) are plugged into the **base plate**, which connects to the **TTFields generator** through a **flat cable.**

inovitro Software

The inovitro system also includes a computer loaded with the inovitro software application, which:

- controls up to 5 TTFields generators in parallel through a USB hub
- enables the user to set **target temperatures** and **frequencies** for each ceramic dish on each base plate
- displays real-time values of parameters

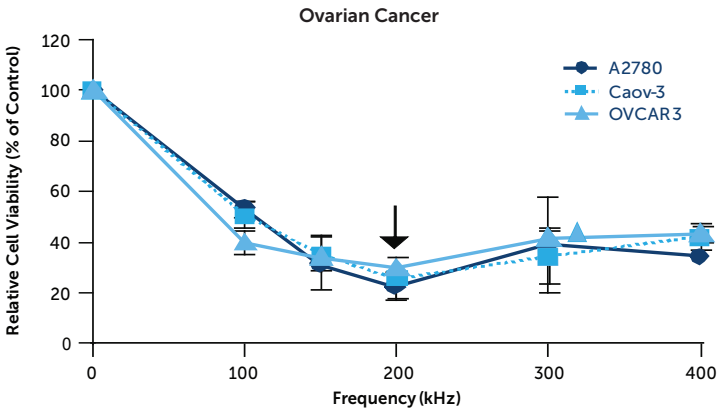


Standard laboratory equipment required: a cooled CO_2 incubator for TTFields application, control CO_2 incubator, biological hood, autoclave, standard cell culture lab equipment, and grounded sockets for TTFields generators and computer. Standard laboratory equipment is not included with the inovitro system.

GBM, glioblastoma; TTFields, Tumor Treating Fields.

Representative Assays Using the inovitro System

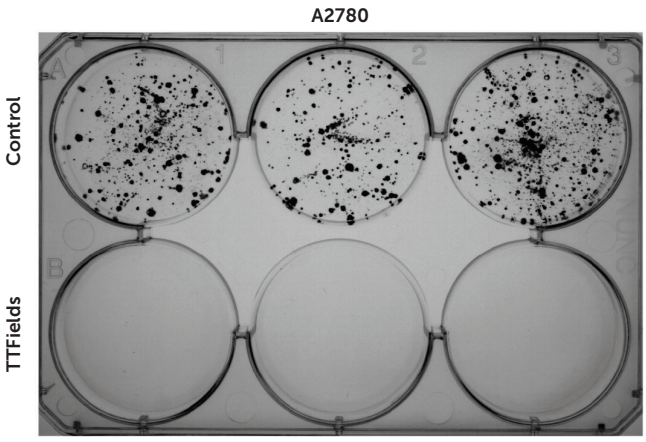
Maximal Inhibitory Effects of TTFields Observed at 200 kHz in Ovarian Cancer Cells[11]



Adapted from Voloshin T et al. 2016

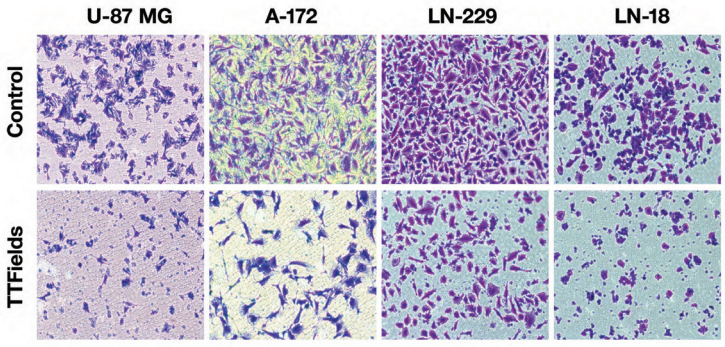
Cell viability assays are used to determine the optimal frequency for cell lines. Ovarian cancer cells were treated for 72 h with TTFields (1.75 V/cm) at different frequencies (100–400 kHz).

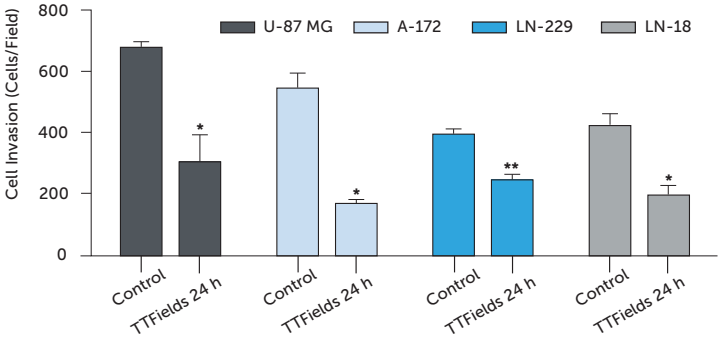
Exposure to TTFields Compromises the Clonogenic Potential of Ovarian Cancer Cells[12]



Clonogenic assay: A2780 cells were plated in triplicate following TTFields application for 72 h at 1.75 V/cm and 200 kHz or untreated (control). Cells were maintained in 37°C for 2 weeks and stained with crystal violet.

Exposure to TTFields Inhibits the Invasive Properties of Cancer Cells[6]





Adapted from Schneiderman RS et al. 2017

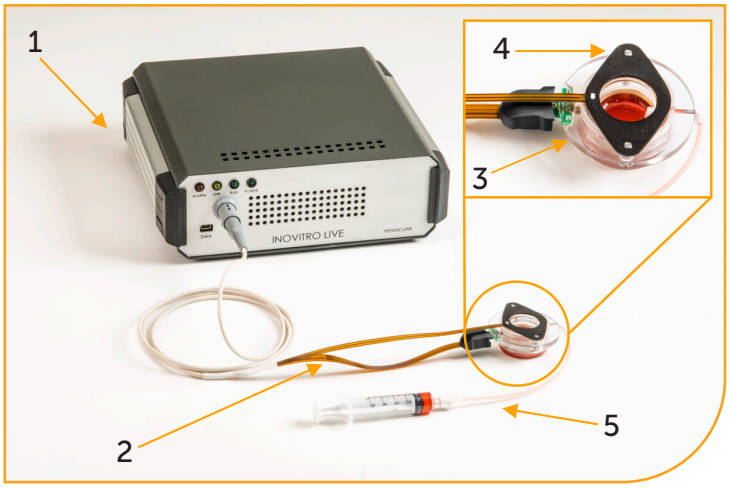
An invasion assay was performed using a modified Boyden chamber (Discovery Labware, Inc. Bedford, MA, USA) placed inside a special high-wall TTFields cell culture dish. Cells were stained with 0.5% crystal violet after 24 h incubation. Cell invasion: Mean + standard error of mean; *P < 0.05, **P < 0.01 from control group, student's t-test.

kHz, kilohertz; TTFields, Tumor Treating Fields.

INOVITRO™ live

TTFields Time-Lapse Microscopy System

The inovitro™ live system enables researchers to perform live cell imaging on cells being treated with TTFields *in vitro*.[10]



inovitro live System Components

1. TTFields generator
2. inovitro live split cable
3. inovitro live ceramic cylinder insert
4. Cover heating element
5. Flexible tube

The basic unit of the system is a high-capacitance **ceramic cylinder insert** with 4 printed electrodes, compatible with a high-wall glass-bottom petri dish.

Flexible tubing connects the petri dish to a sterile syringe for media replacement. The **TTFields generator** is connected to the ceramic cylinder insert and the **cover heating element** through a **split cable**.

The inovitro live insert is inserted into the high-wall glass-bottom petri dish.

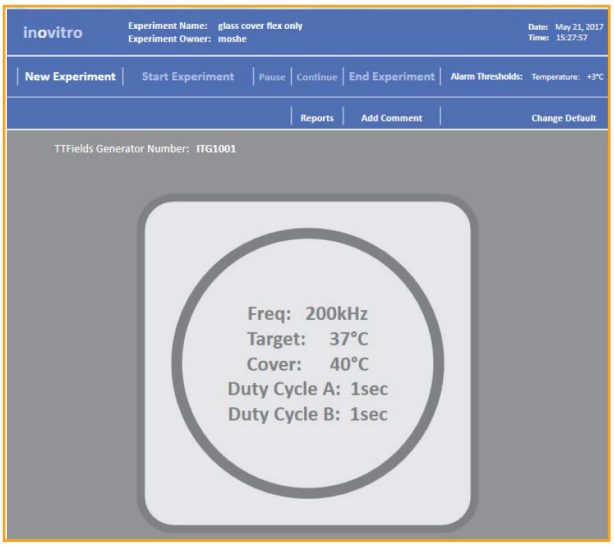
The inovitro live cable is connected to the insert.

The cover heating element with temperature control is secured over the inovitro live insert.

inovitro live Software

The inovitro live system also includes a computer loaded with the inovitro live software application, which:

- controls 1 TTFields generator, connected through a mini USB cable
- enables the user to set **target temperature** and **frequency** to be applied to the cells and to control directionality of the applied field
- displays real-time values of parameters

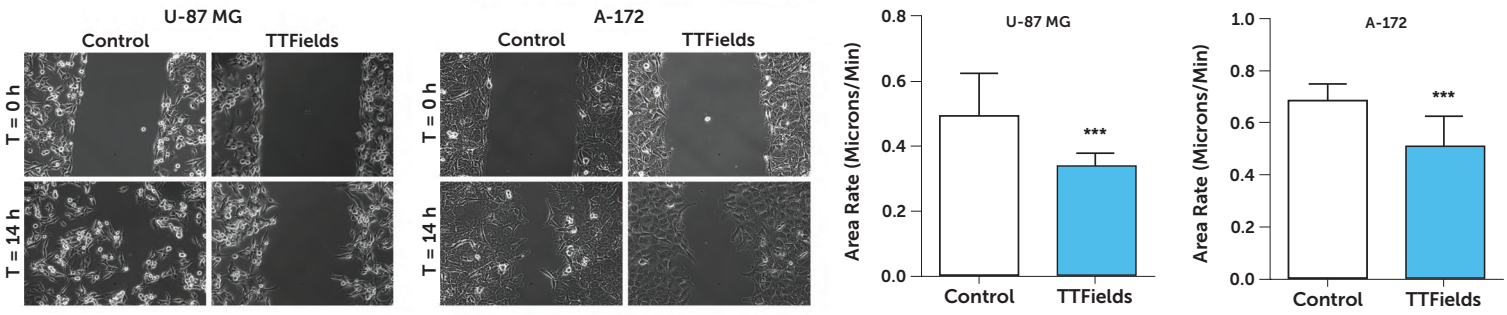


Standard laboratory equipment required: inverted microscope equipped with a CO_2 incubator to allow for ambient temperature of 24°C, biological hood, autoclave, ibidi 35 mm high-wall glass-bottom dish, ibidi glass cover for 35 mm dish, standard cell culture lab equipment, and grounded sockets for TTFields generator and computer. Standard laboratory equipment is not included with the inovitro live system.

TTFields, Tumor Treating Fields.

Representative Assays Using the inovitro live System

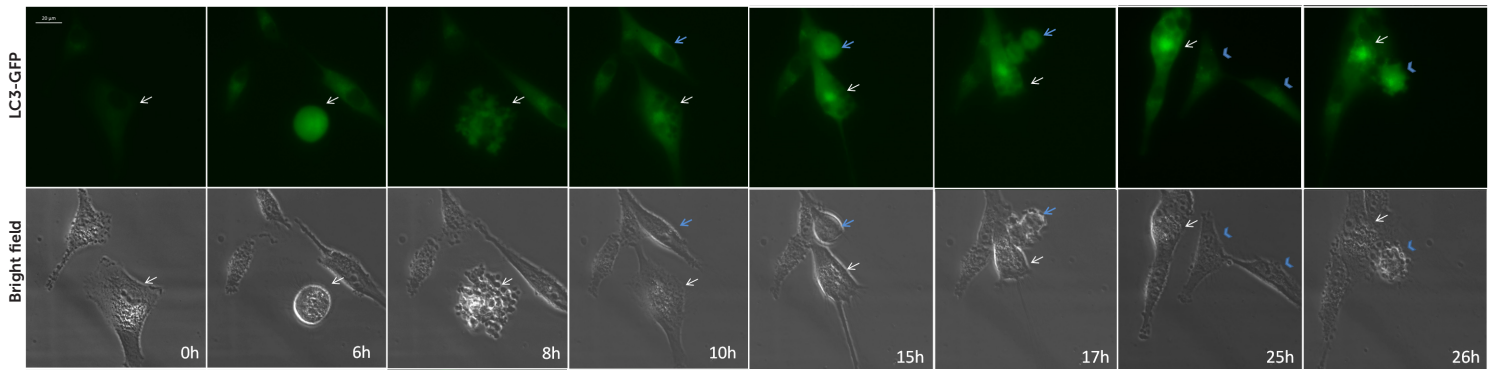
Exposure to TTFields Reduces the Migration Rates of Glioma Cells[13]



Adapted from Schneiderman RS et al. 2016

Wound-Healing Assay: Migration was observed in time-lapse series for 24 h (Zeiss axio observer [Carl Zeiss Microscopy GmbH, Jena, Germany]; 10X objective). Phase contrast images were collected every 12 min. Area rate: Mean + standard error of mean; ***$P < 0.001$.

Exposure to TTFields Induces Upregulation of Autophagy in Glioma Cells[7]



Adapted from Shteingauz A et al. 2018

Autophagy Assessment Assay: U-87 MG cells stably expressing LC3-GFP and treated with TTFields were followed using time-lapse microscopy for 26 h (Zeiss axio observer [Carl Zeiss Microscopy GmbH, Jena, Germany]; 10X objective) for LC3 upregulation and mitotic state assessment. Arrows indicate cells entering mitosis and arrowheads denote the resulting daughter cells.

GFP, green fluorescent protein; TTFields, Tumor Treating Fields.

Working With inovitro™ and inovitro™ live Systems

The Optimal TTFields Frequency for Disrupting Mitosis *In Vitro* Is Cell Type-Specific and Inversely Related to Cell Size[3,*]

Tissue	Disease	Cell Line(s)	Optimal Frequency (kHz)
Brain	GBM	A172, DKMG, LN18, LN229, U87, U118MG, U251MG	200
Buccal mucosa	Squamous cell carcinoma	H157	100
Colon	Colorectal carcinoma	HCT15	150
Kidney	Renal cell adenocarcinoma	7860	240
Liver	Hepatocellular carcinoma	C3A, Huh-7D12	150
Lung	Adenocarcinoma	HCC-827	150
	Mesothelioma	MSTO-211H	150
	NSCLC adenocarcinoma	HCC-4006, A549	150
	NSCLC carcinoma	NCI-HI299	150
	Squamous cell carcinoma	NCI-H520	150
	SCLC carcinoma	DMS114, NCI-H2052	200
Mammary gland; breast	Breast adenocarcinoma	MCF-7, MDA-MB-231	150
Ovary	Ovarian adenocarcinoma	A2780, CAOV3, NIHOVCAR	200
Pancreas	Pancreatic adenocarcinoma	AsPC-17[4]	150
Skin	Malignant melanoma	A375, Sk-Mel2, MEWO	150
Stomach	Gastric adenocarcinoma	AGS	150
	Gastric carcinoma	KATOIII	150
Urinary Bladder	Carcinoma	5637	150
Uterus	Uterine sarcoma	MES-SA	100

*Use table as a reference; specific frequency should be determined for each individual cell type and experimental conditions.

Outcomes After TTFields Application Can Be Quantified Based on Standard Assays With Differences Between the inovitro and inovitro live Systems

INOVITRO™		INOVITRO™ live
50–500	Frequency range (kHz)	50–500
8–40	Number of dishes	1
✓	Microscopy	✓
×	Time lapse microscopy	✓
✓	Cell counts	✓
×*	Migration	✓
✓†	Invasion	×
✓	DNA/RNA/protein extraction	✓‡
✓	Flow cytometry	✓

Standard laboratory equipment required. *Possible as endpoint, not as time-lapse; †Using high-wall dish; ‡Limited amount.

GBM, glioblastoma; kHz, kilohertz; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

INOVIVO™
TTFields *In Vivo* System

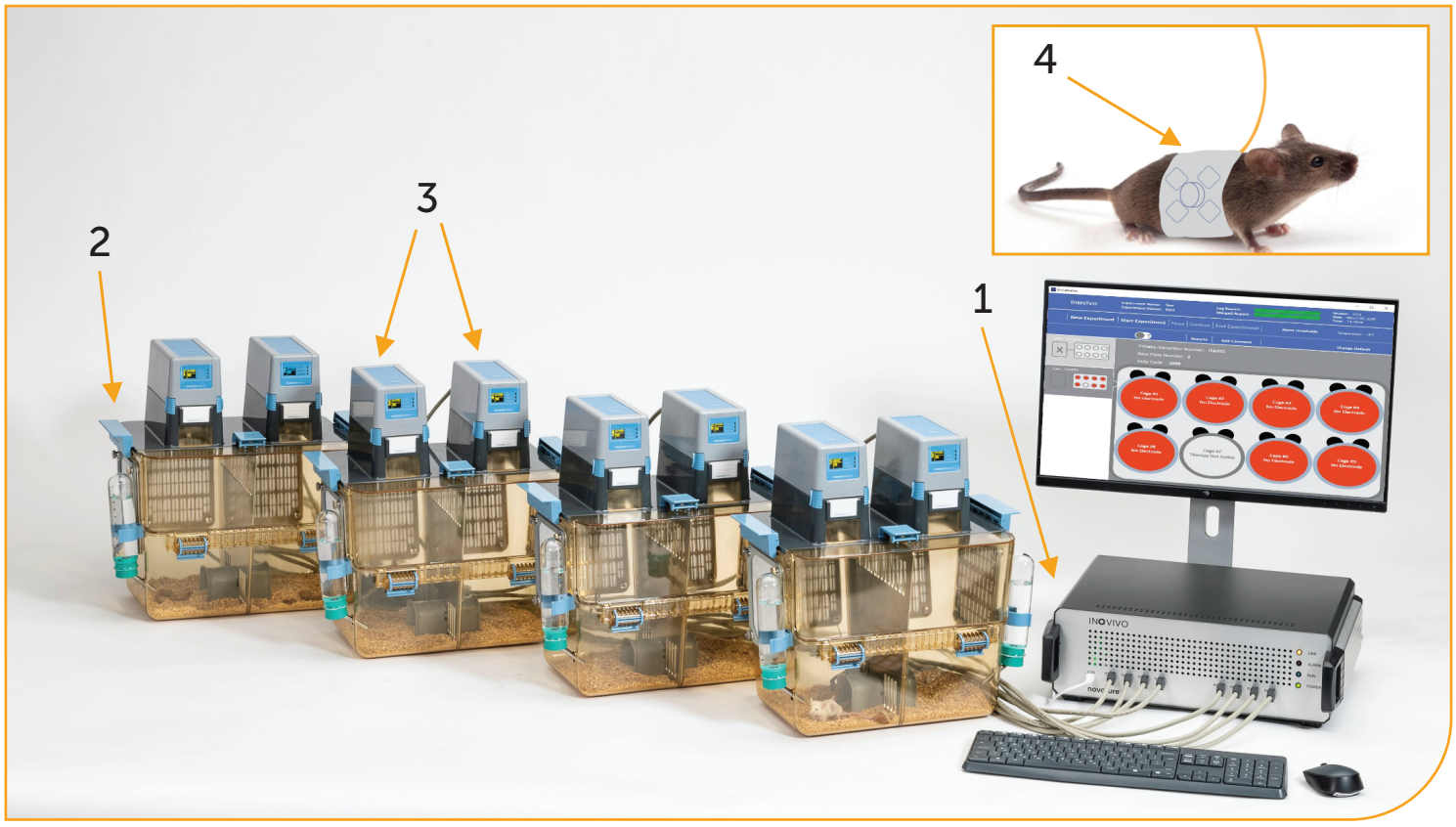
The inovivo™ system is a preclinical laboratory research system that enables researchers to study the effects of TTFields in animal tumor models.

The system is designed to apply TTFields to subcutaneous and/or orthotopic tumors located in the thorax/abdomen of mice or other small rodents with an intact immune system.

inovivo System Components

1. TTFields generator
2. Cages
3. Swivel system
4. Transducer arrays

Representative Example of the inovivo System Setup With 4 Cages



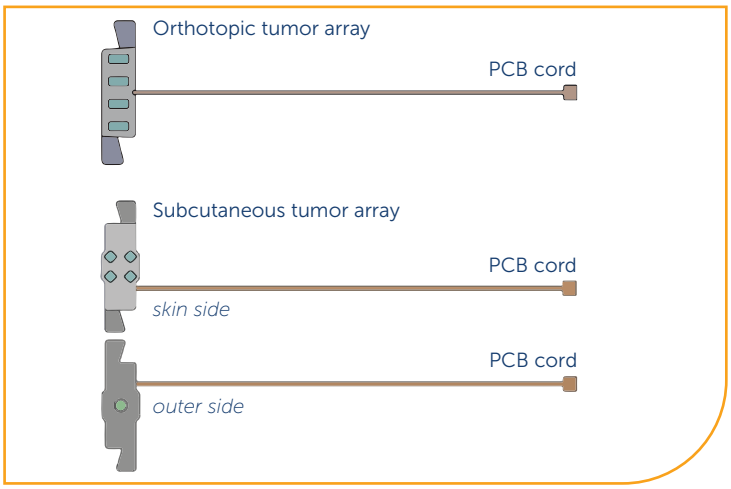
There are 4 **cages** per inovivo system, designed to house 2 mice each, with a minimal barrier between the animals.

PCB, printed circuit board; TTFields, Tumor Treating Fields.

INOVIVO™
TTFields *In Vivo* System

The **swivel system** is a motorized system with a motion sensor and is connected to the flex **PCB cord** that delivers TTFields to the mouse. It is designed to prevent entanglement of the mouse and twisting and breakage of the PCB cord by reacting to the movements of the animal and rotating the connector to the transducer array accordingly.

The **transducer arrays** are composed of ceramic transducers and flex PCB to deliver TTFields. They are adhesive and are placed directly to the shaved thorax/ abdomen of the animal to deliver TTFields to the tumor. There are 2 types of transducer arrays: one designed for treating orthotopic tumors and one designed for treating subcutaneous tumors.



The inovivo Software Application

The inovivo system also includes a computer loaded with the inovivo software application, which:

- displays real-time values of parameters, allowing the user to control the experimental parameters and to monitor experiment progress of a single inovivo generator
- allows control of up to 8 different treatments in parallel through a USB hub
- enables the user to set **target temperatures and frequencies** for TTFields treatment or choose heat mode for control treatment

Standard laboratory equipment required: standard equipment for housing and anesthetizing mice, timer, depilatory cream, polydine solution, grounded sockets for TTFields generators and computer. Standard laboratory equipment is not included with the inovivo system. All animal studies must be conducted in compliance with the Institutional Animal Care and Use Committee.

PCB, printed circuit board; TTFields, Tumor Treating Fields.

NOVOCURE SUPPORTS INDEPENDENT RESEARCHERS AS THEY ADVANCE BASIC AND TRANSLATIONAL RESEARCH ON TTFields BY

Providing the inovitro™, inovitro™ live, and/or inovivo™ Systems Based on a Grant Application

Novocure reviews grant applications on a continual basis. If approved, Novocure will assist with delivery and installation of systems in your lab. Training will be done within your facility or at the Novocure R&D facility in Haifa, Israel.
Grant experiments will be conducted in grantee lab.

Researchers are invited to request additional information or inquire about the proposal submission process and how to gain access to a system for your lab by contacting: inovitro@novocure.com

Partnering With AACR to Award the AACR-Novocure TTFields Research Grants

These grants have been awarded to investigators worldwide who are conducting innovative research on TTFields. Basic and translational research focused on the preclinical application of TTFields is supported by this program.

For more information, please visit www.aacr.org or contact one of our representatives.

These grant programs are intended to provide a deeper understanding of the mechanism of action of TTFields and to accelerate the development of new treatment strategies to advance therapeutic options for cancer.

References

1. Optune [instructions for use]. Portsmouth, NH: Novocure; January 2019. **2.** NovoTTF-100L [instructions for use for Unresectable Malignant Pleural Mesothelioma]. Portsmouth, NH: Novocure; May 2019. **3.** Lavi-Shahaf G et al. *Cancer Res.* 2019;79(suppl 13): Abstract 1258. As presented at the American Association for Cancer Research (AACR) Annual Meeting; March 29-April 3, 2019; Atlanta, GA. **4.** Giladi M et al. *Sci Rep.* 2015;5:18046. **5.** Giladi M et al. *Radiat Oncol*. 2017;12(1):206. **6.** Schneiderman RS et al. Neuro-Oncol. 2017;19(suppl 6): Abstract CBIO-30. As presented at the Society for Neuro-Oncology (SNO) Annual Meeting; April 16-19, 2017; San Francisco, CA. **7.** Shteingauz A et al. *Cell Death Dis*. 2018;9(11):1074. **8.** Voloshin T et al. [published online ahead of print March 6, 2020]. *Cancer Immunol Immunother.* 2020. doi: 10.1007/s00262-020-02534-7. **9.** inovitro™ [instructions for use]. Haifa, Israel: Novocure, November 2015; Novocure Data on File QSD-QR-800. **10.** inovitro™ live [user manual]. Haifa, Israel: Novocure, October 2017; Novocure Data on File QSD-QR-707. **11.** Voloshin T et al. *Int J Cancer.* 2016;139(12):2850-2858. **12.** Schneiderman RS et al. *Cancer Res.* 2014;74(suppl 19): Abstract 5521. As presented at the American Association for Cancer Research (AACR) Annual Meeting; April 5-9, 2014; San Diego, CA. **13.** Schneiderman RS et al. *Cancer Res.* 2016;76(14 suppl): Abstract 5078. As presented at the American Association for Cancer Research (AACR) Annual Meeting; April 16-20, 2016; New Orleans, LA.