PMWC 201

PRECISION MEDICINE WORLD CONFER

Immune System Key Personalized Novel Immunotherapy

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About Us

History and Status

- Immune System Key Ltd. (ISK) was founded in 2005
- The company is developing drug for treatment of life threating diseases : AML, Hi-Risk MDS and TNBC

- The company has successfully completed Phase I is solid tumor patients(“all comers”) , in order to start ASAP phase II hematology clinical trial and solid tumor trial.

Nerofe - Background

What is Nerofe?

Nerofe-novel human peptide hormone- has been isolated and cloned by ISK

Nerofe™ or "human Thymus-Expressed Apoptosis Factor" (hTEAF), ISK's flagship compound, has been found to have strong anti-cancer activity.

It has been modified to a 14-amino acid form of a novel human hormone-peptide, a native ligand of the ST2 receptor, which plays a pivotal role in immune system response(ST2 is mainly expressed on NK cells and DC cells)

Applying Nerofe to ST2 receptor over expressing human cancer cells, induces Golgi destruction and cancer cell death (accompanied with BiP secretion from dead cancer cells- great tool for prediction of successful treatment)

Natural Nerofe

- Is expressed in human thymus as 84 AA peptide
- Has a very strong signal peptide (mature peptide is 51 AA)
- Has a human plasma level of 800pg/ml
- Is found in several species including the mouse
- Binds to ST2 receptor and selectively induces apoptosis in cancer cells due to over expression of ST2 in cancer cells

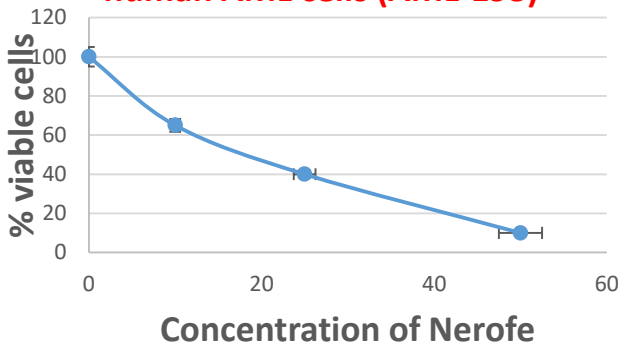
API Nerofe

- API Nerofe™ is a stabilized 14 AA peptide
- The API and DP are GMP manufactured
- Full GLP toxicology studies in beagles and rats
  - Dog (MTD) – 80mg/kg, (NOEL) – 20mg/kg , (T1/2) – 16hr.
- Safety
  - The peptide is extremely safe for oncology patients– no toxic effects were seen after a 16-fold increase in doses and no side effects were observed.
- IP protection
  - The company holds several worldwide patents, both of the chemical structure of the peptide and its derivatives and of its different uses/indications WO 2006/046239).

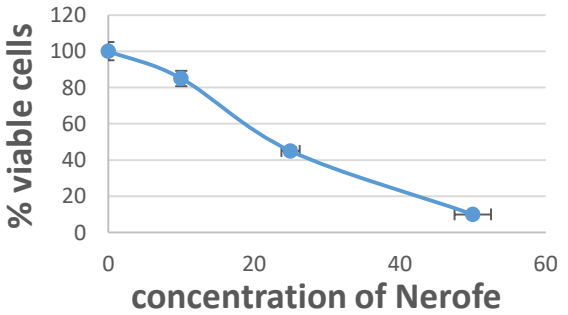
Nerofe™ - an efficient drug candidate for AML

In vitro effect of Nerofe on different human AML cell lines

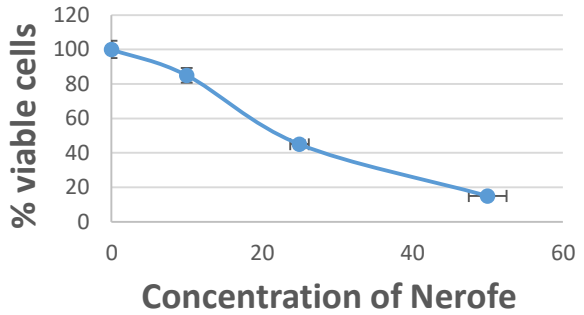
Effect of Nerofe on viability of human AML cells (AML-193)



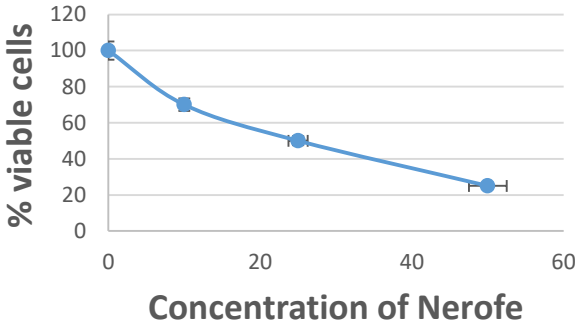
Effect of Nerofe on viability of human AML cells (THP-1)



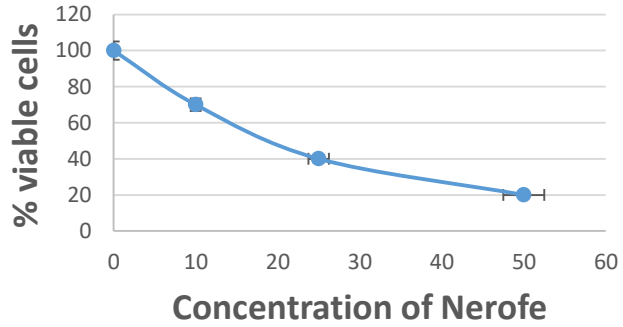
Effect of Nerofe on viability of human AML cells (Kasumi-1)



Effect of Nerofe on viability of human AML cells (U937)

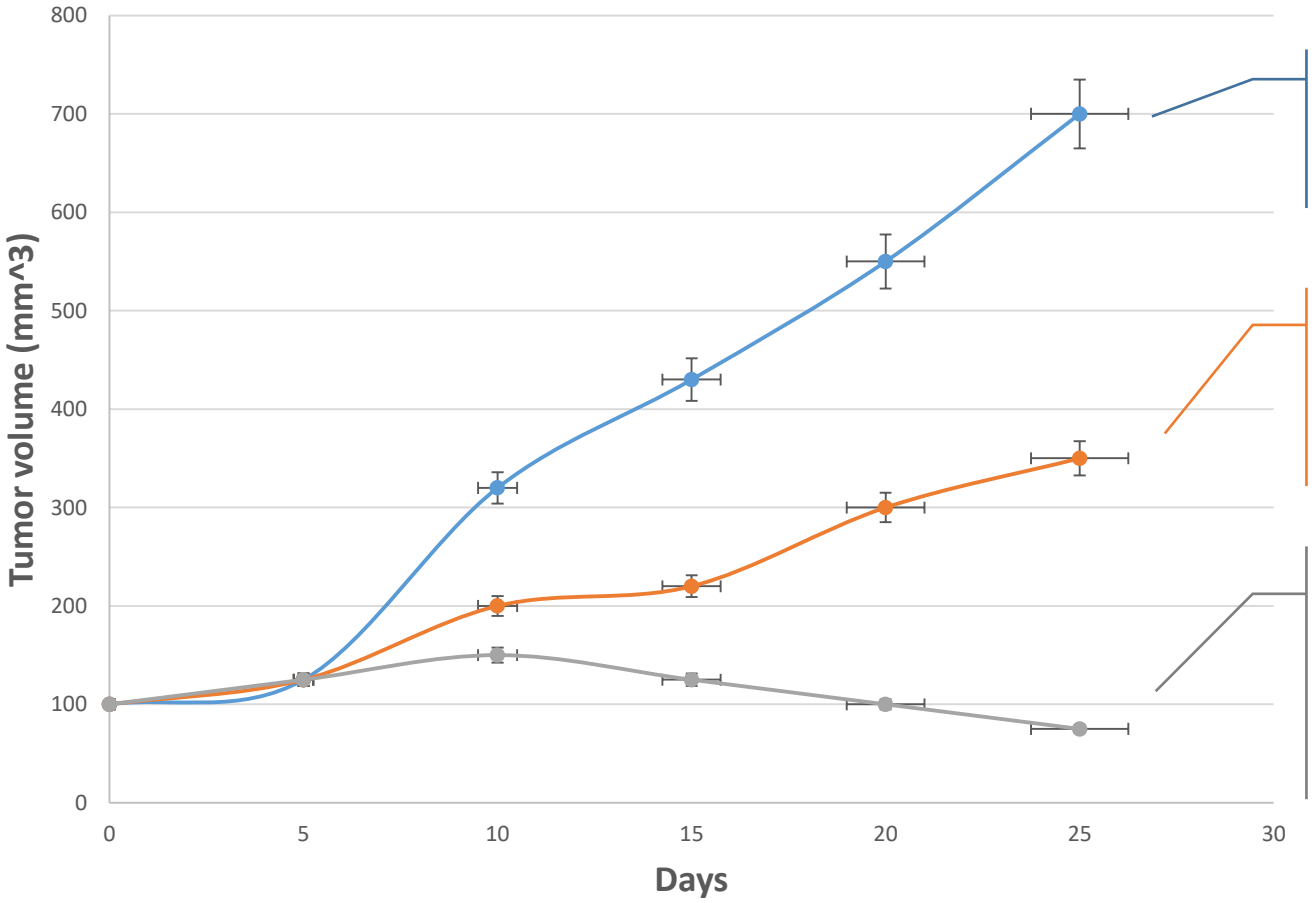


Effect of Nerofe on viability of human AML cells (ML2)



Nerofe™ - an efficient drug candidate for AML

Effect of Nerofe on ML2 tumors in nude mice



Tumor volume of mice treated with 5% mannitol.

Tumor volume of mice treated IP with X mg/Kg of Nerofe 3 times a week.

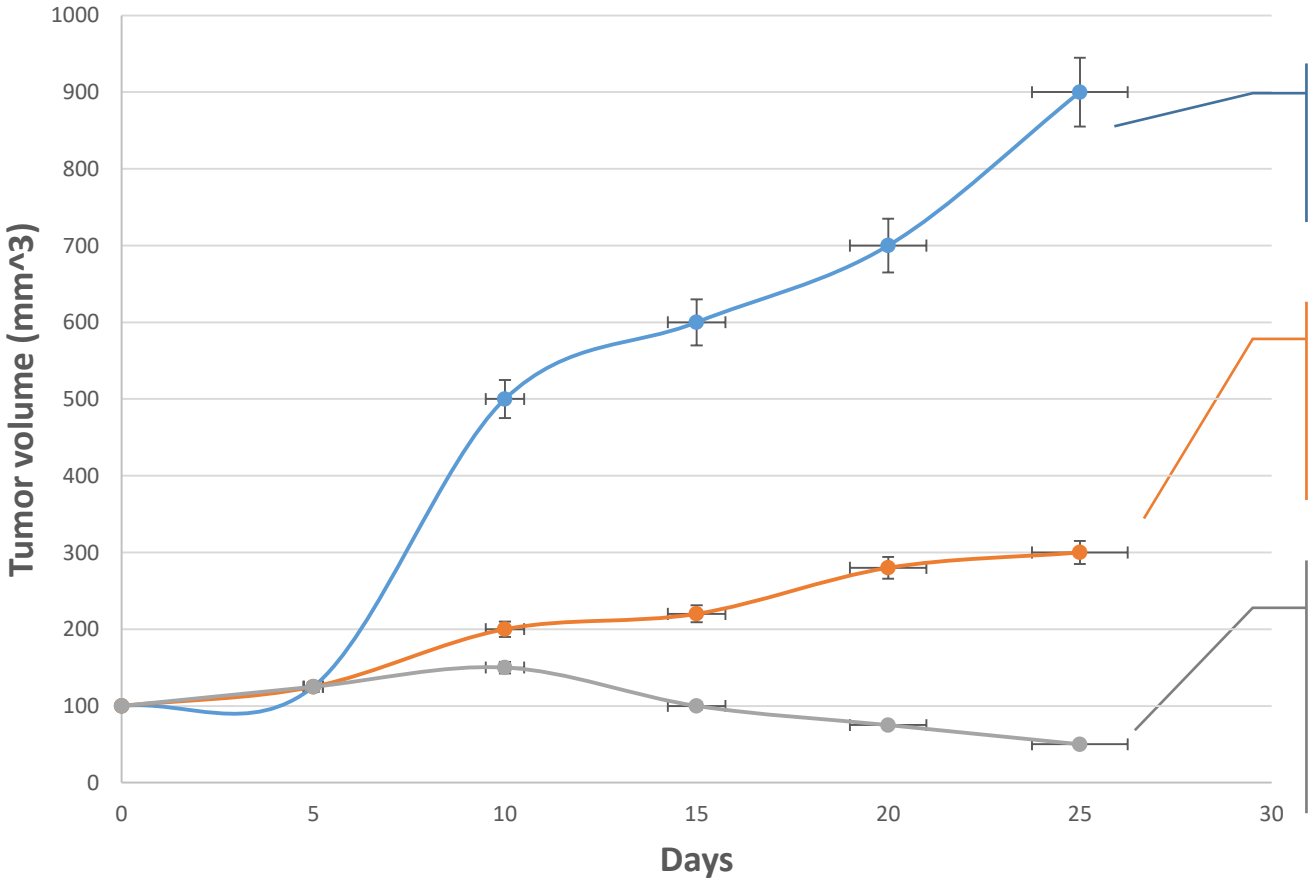
Tumor volume of mice treated IP with 3*X mg/Kg of Nerofe 3 times a week. (experiment repeated twice)

15 million ML2 cells were inoculated SC in nude mice (7 mice per group).

Mice entered the trial after tumor reached volume of 100mm^3

Nerofe™ - an efficient drug candidate for AML

Effect of Nerofe on THP-1 tumors in nude mice



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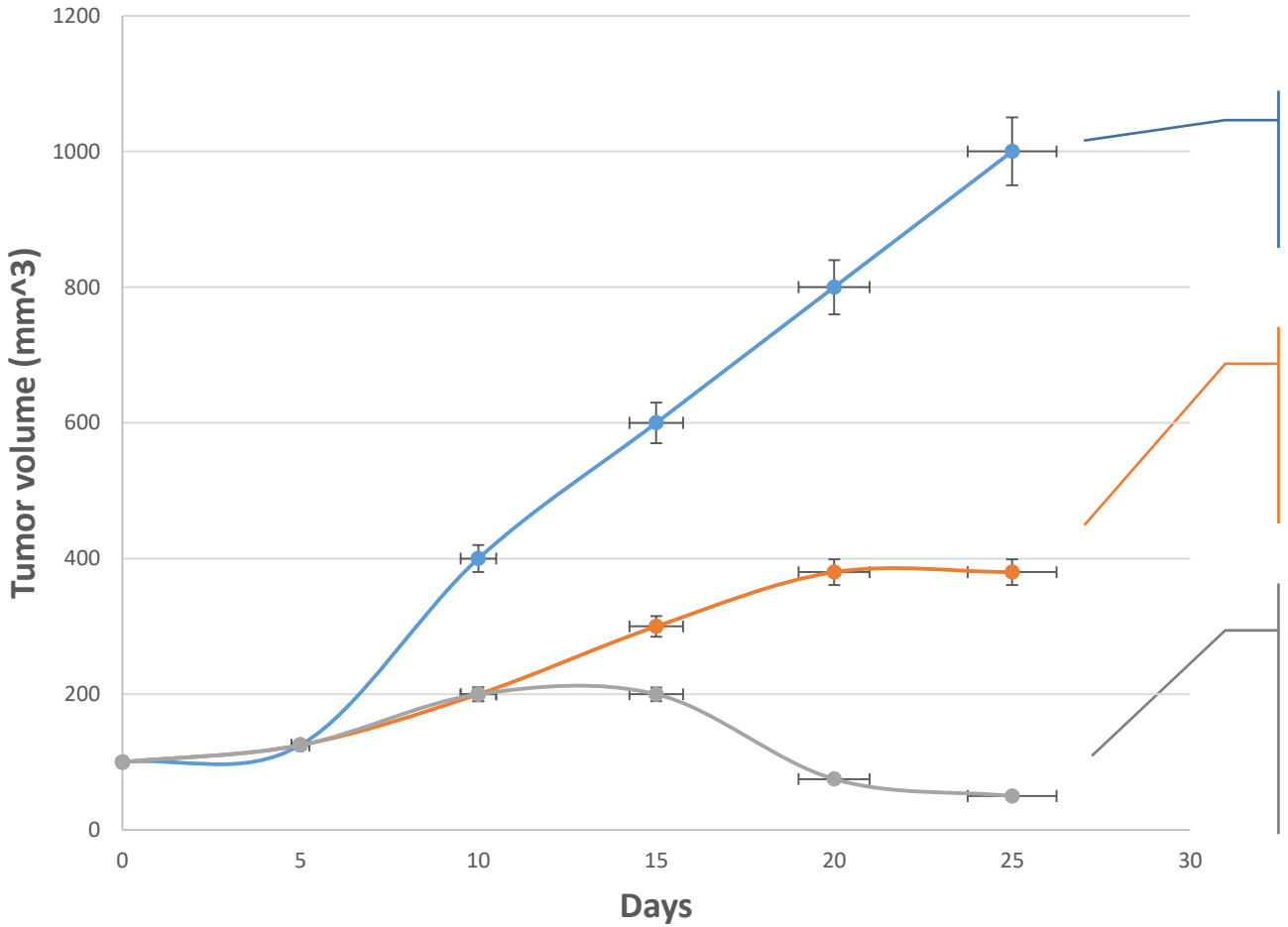

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Nerofe™ - an efficient drug candidate for AML

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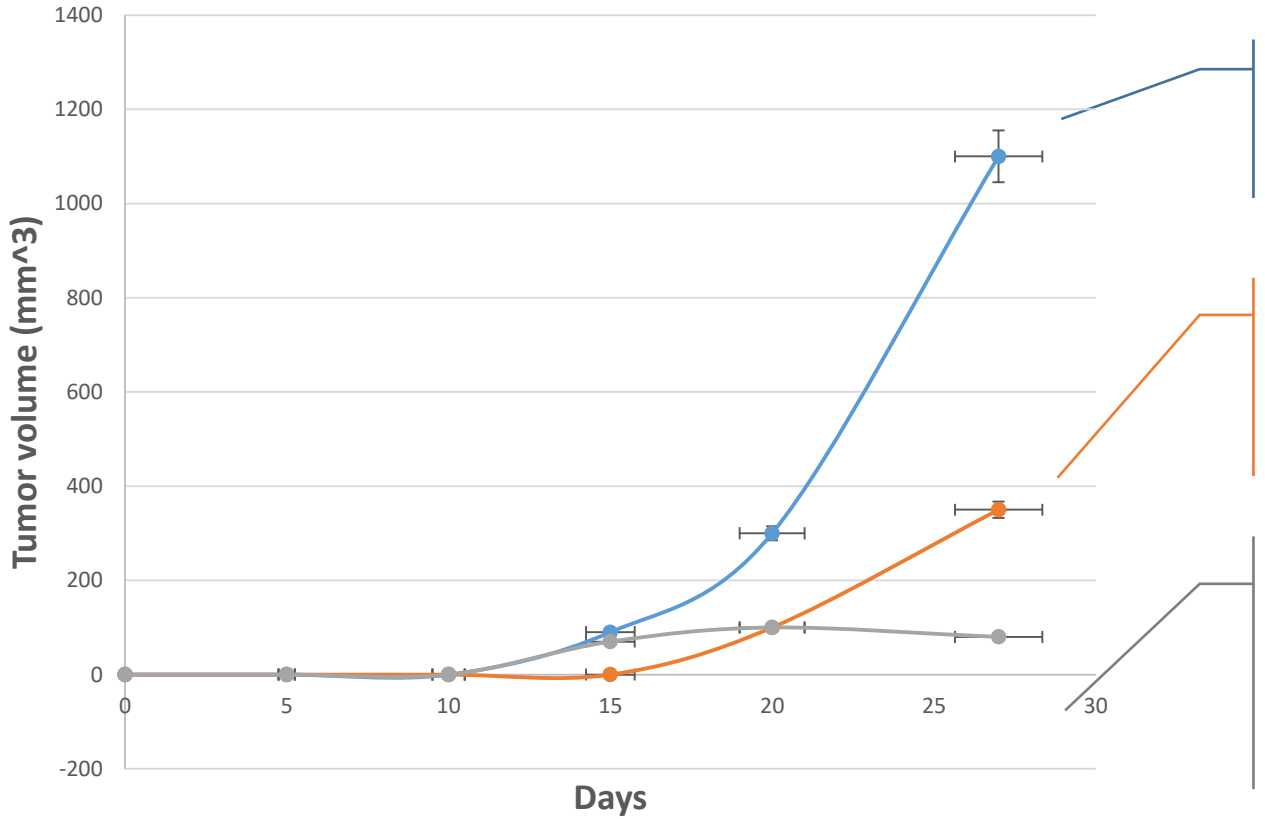

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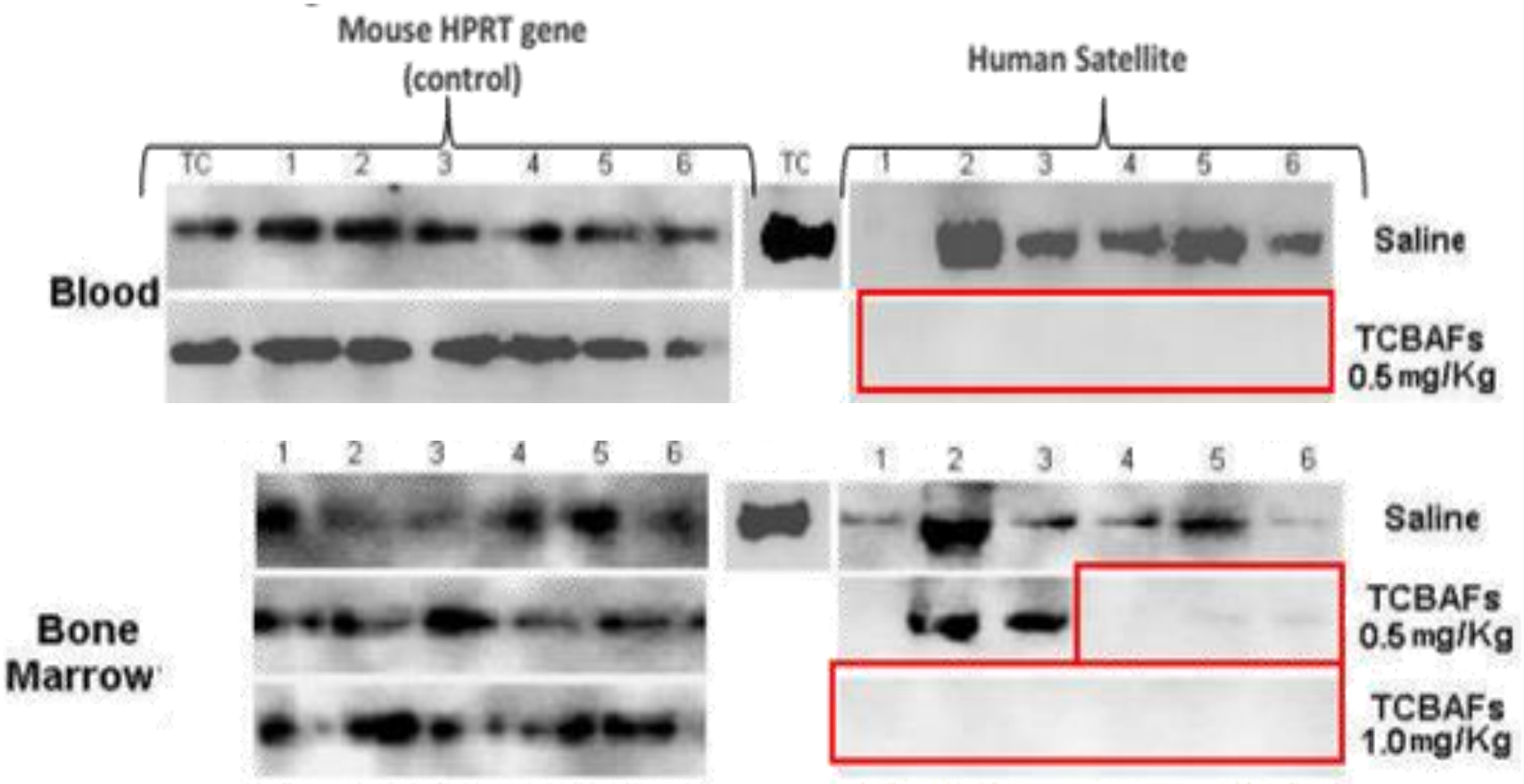
15 million ML2 cells were inoculated SC in nude mice (7 mice per group).

Mice entered the trial after tumor reached volume of 100mm^3

Nerofe™ - an efficient drug candidate for AML

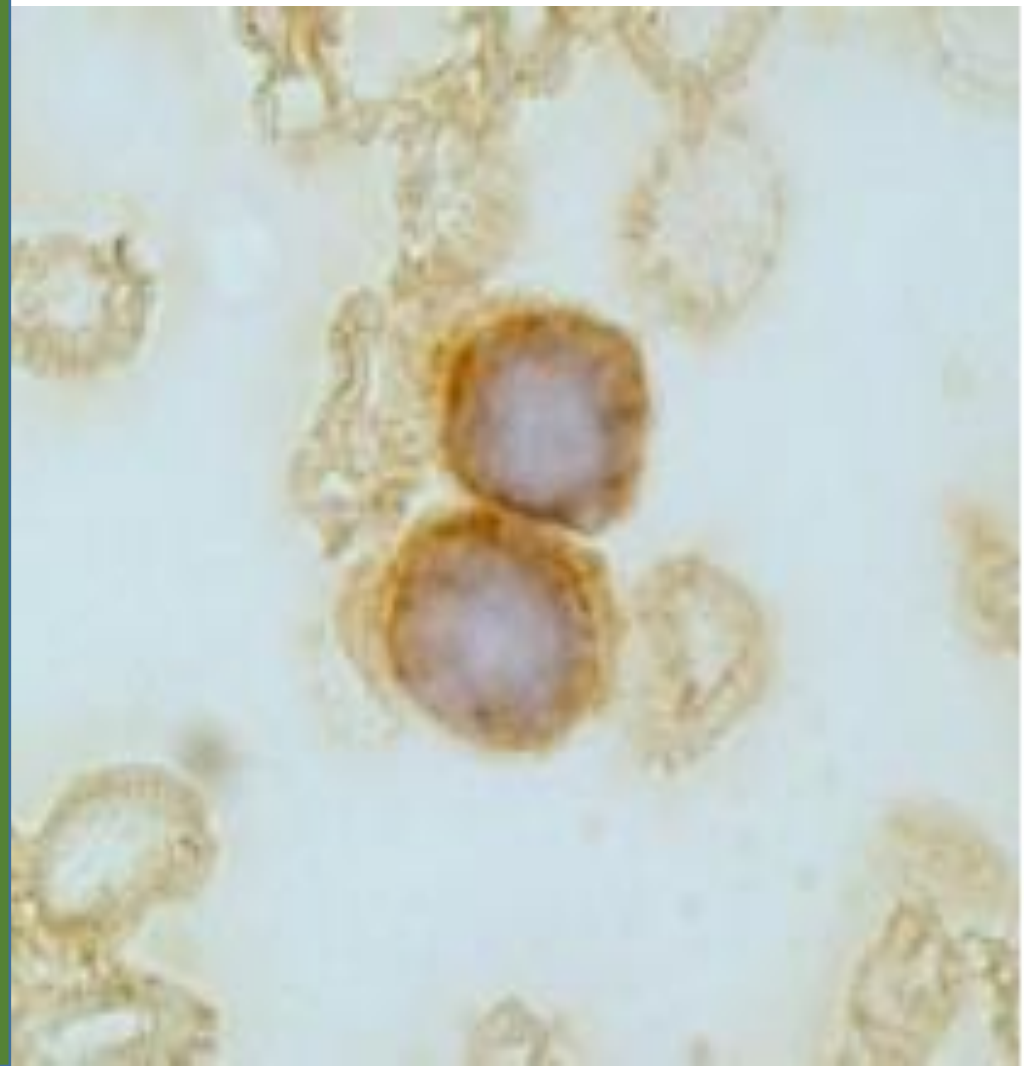
Currently the only known drug able to totally eliminate cancer cells from bone marrow

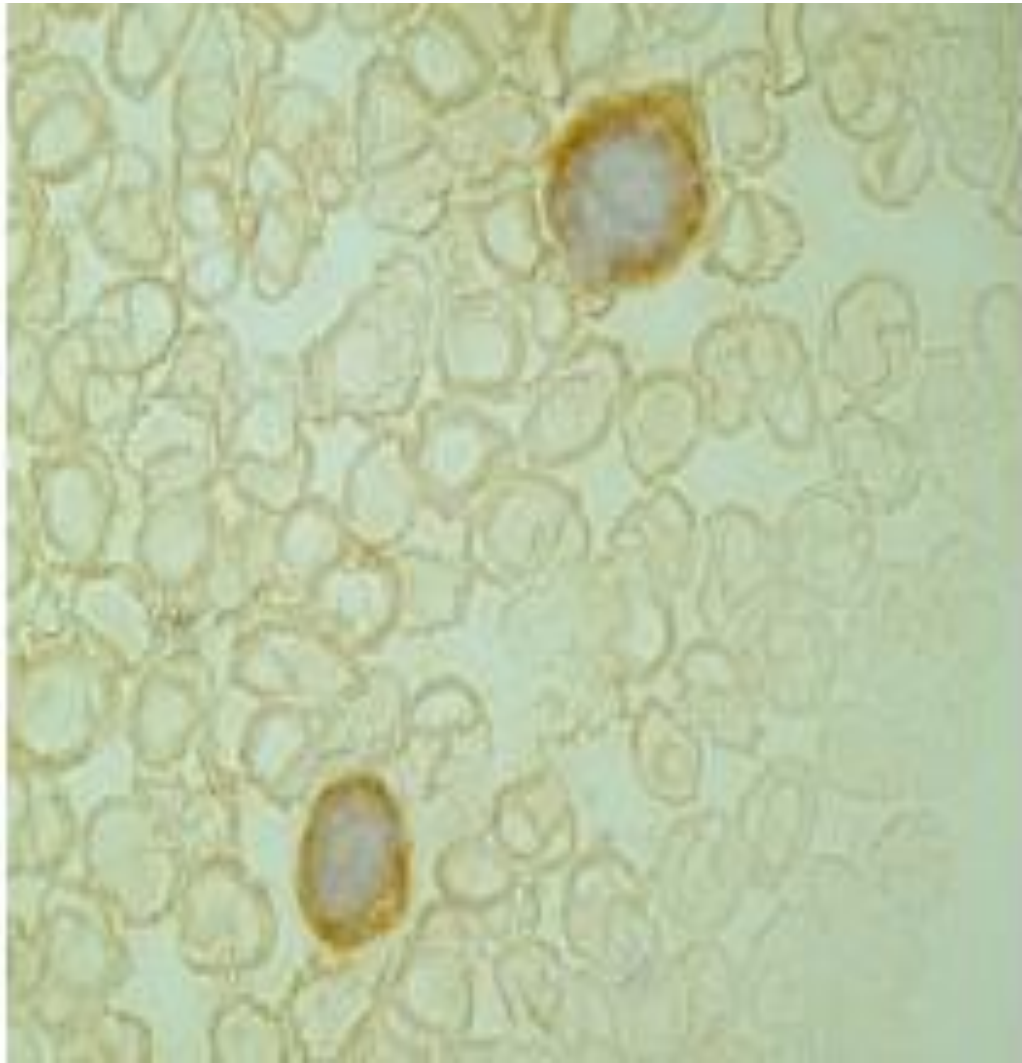


Mice were injected with human AML cells (U937 cell line), once cells penetrated bone marrow mice started IP administration treatment with different doses of Nerofe vs. saline (control group). Detection of human cells in blood and bone marrow was done with RT-PCR looking at human satellite(40 cycles). We can see clearly that Nerofe caused complete disapprence of human AML cells from bone marrow and blood in a dose depended manner.
Same results were obtained with ML2 cell line

*Full length ST2 is over expressed in **MDS** cells*

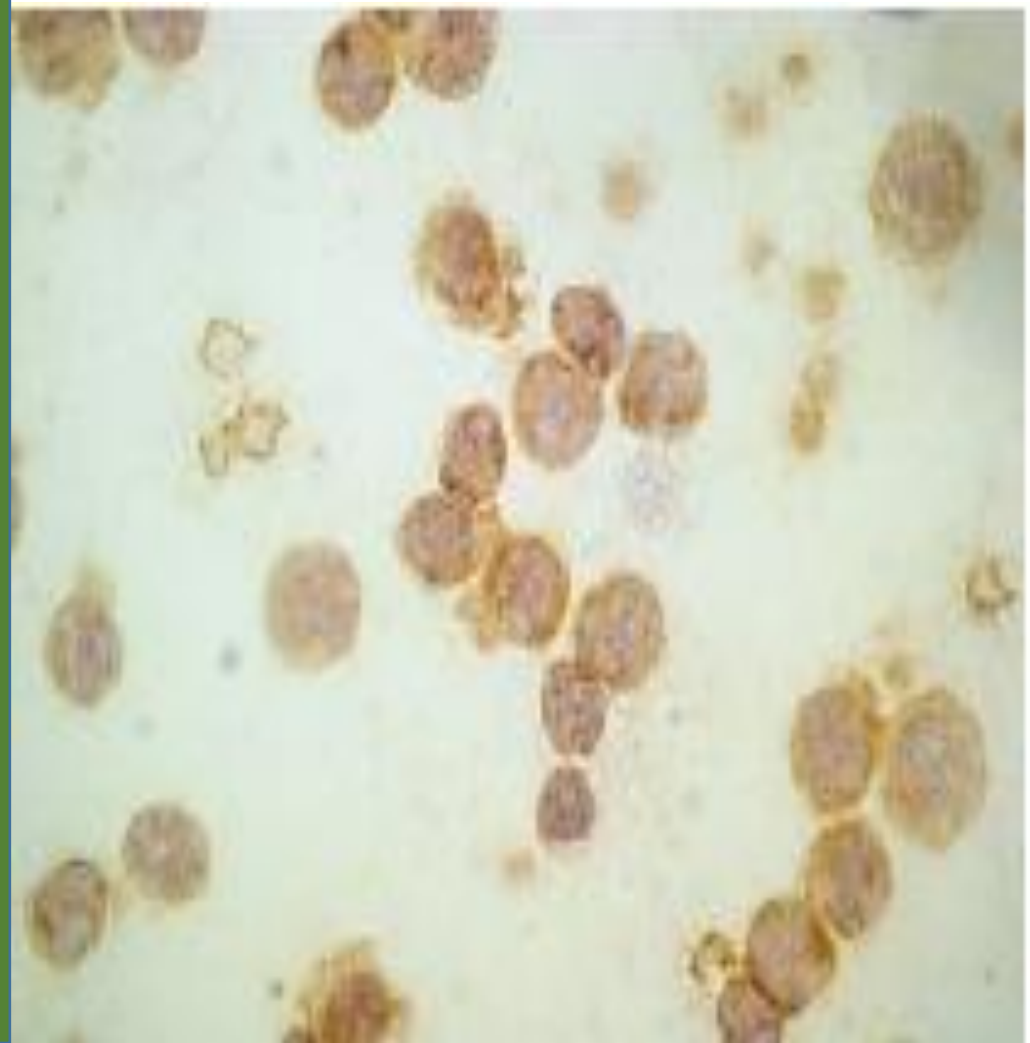
Human biopsies from BM

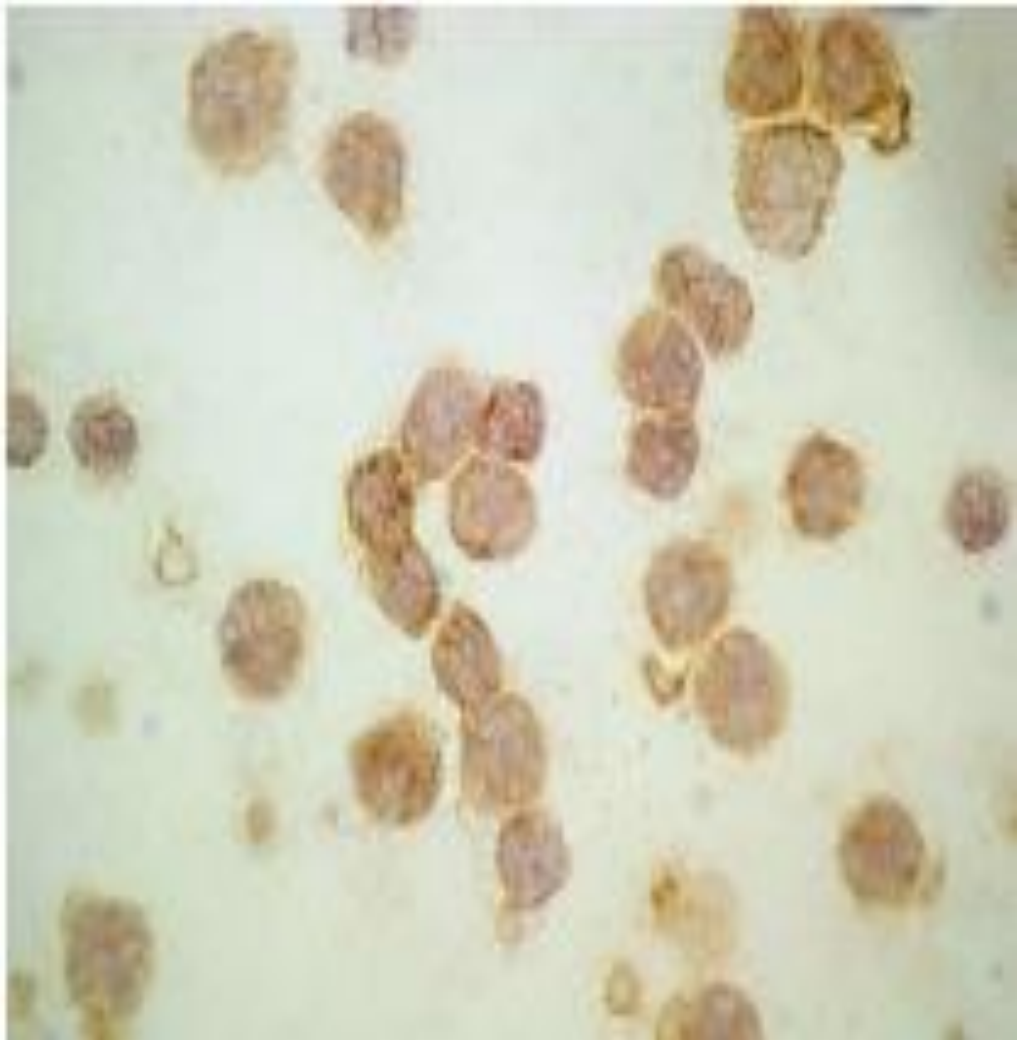




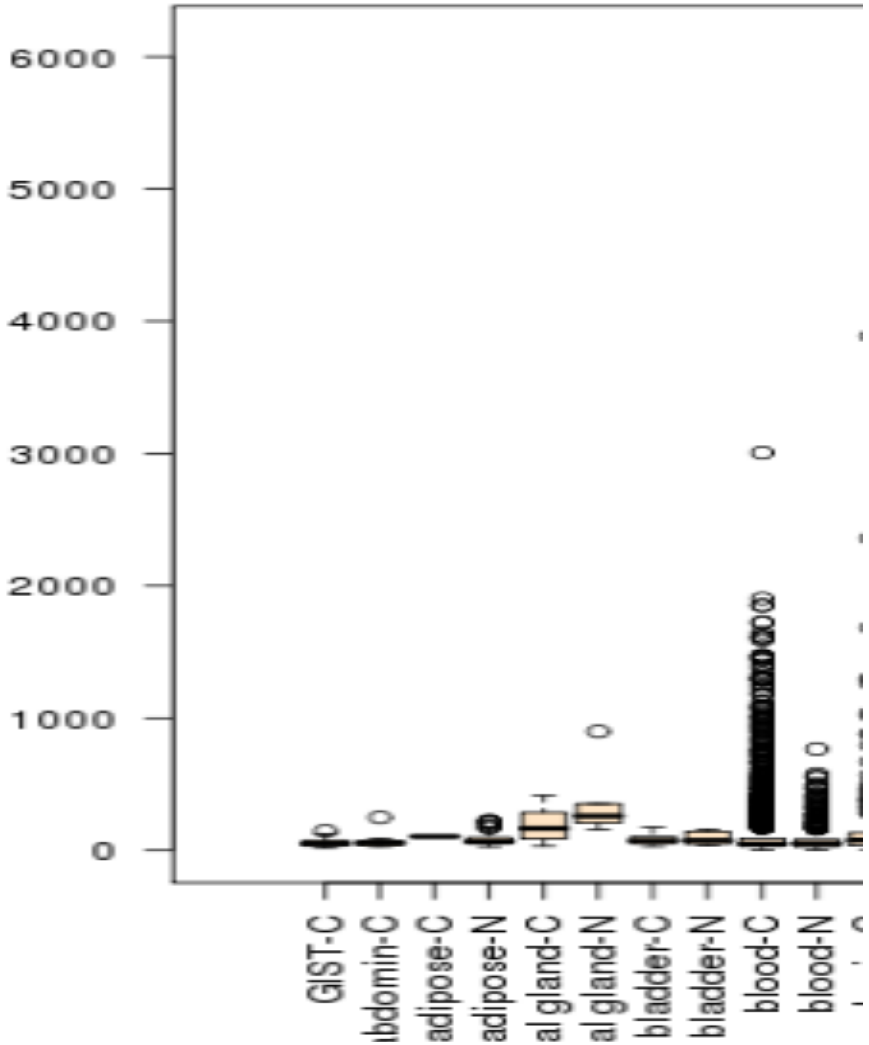
*Full length ST2 is over expressed in **AML** cells*

Human biopsies from BM





Incidence of ST2 expression in AML patients

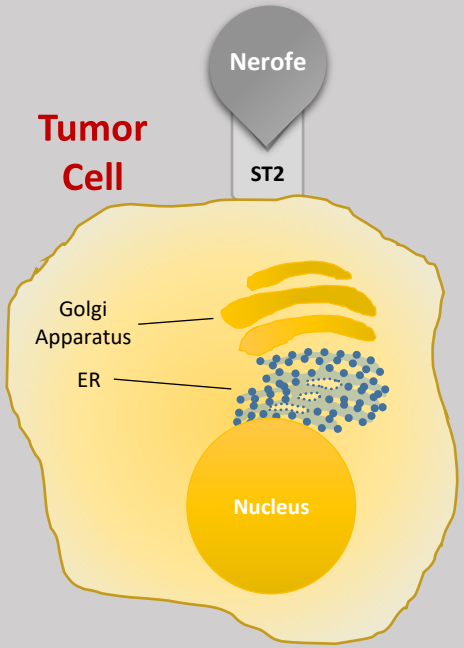


Blood-C Cancer blood
Blood-N Normal blood

Acute myeloid leukemia samples of samples =< 60yrs on HG-U133 plus 2. GEO dataset GSE6891

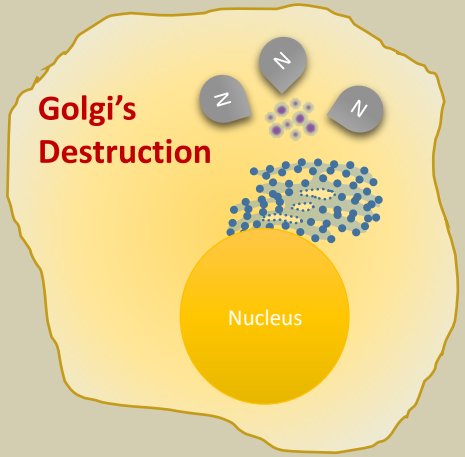
Nerofe's MOA

Nerofe enters the tumor cells through ST2 receptor



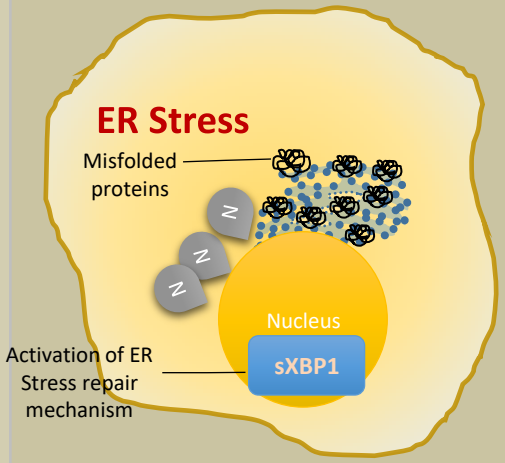
1

Nerofe reaches the Golgi Apparatus and induces it's destruction



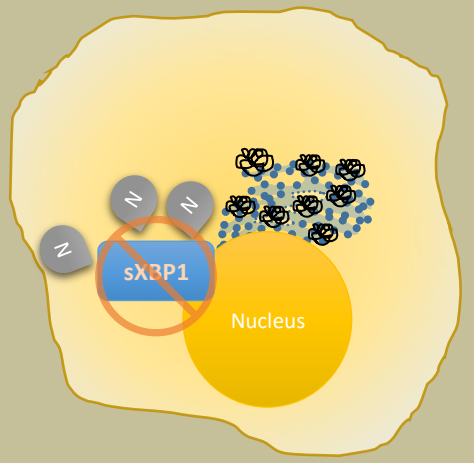
2

Due to Golgi's destruction protein accumulates in the ER which leads to ER stress and activation of ER stress repair mechanism



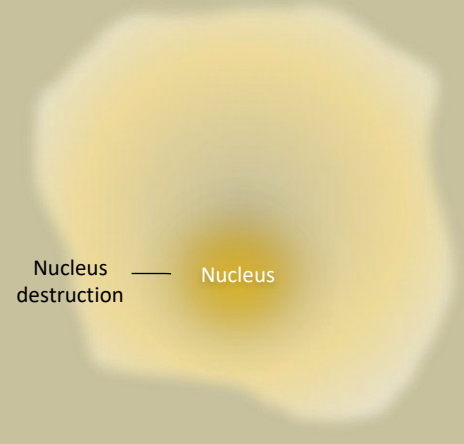
3

ER stress repair mechanism is inhibited by Nerofe's downregulation of sXPB1



4

Cell death caused by unrepaired ER stress



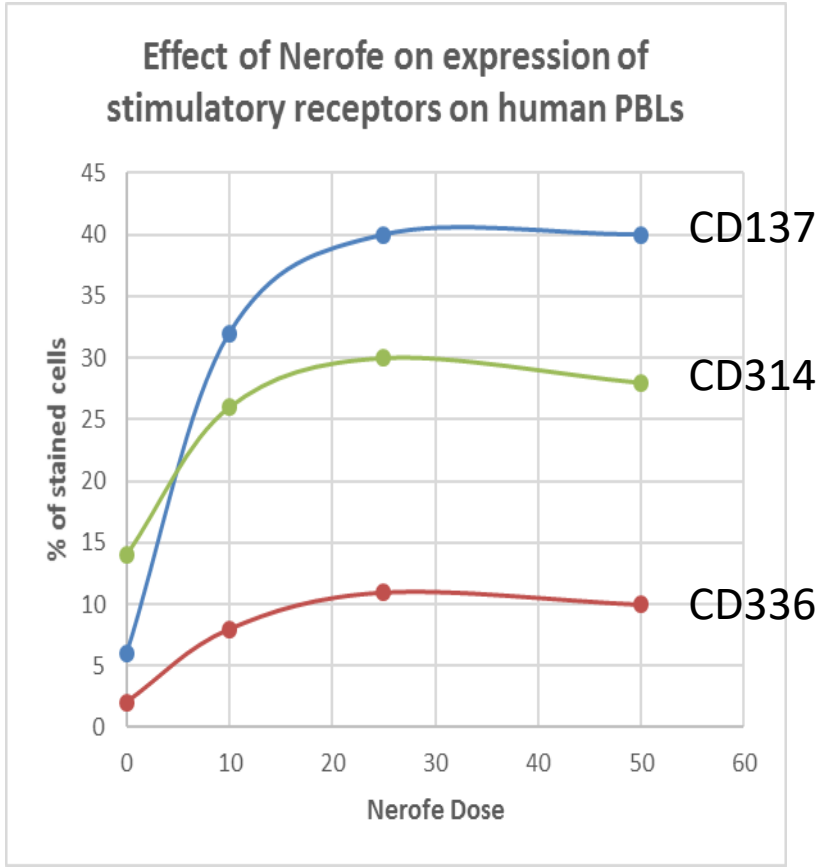
5

Effect of Nerofe on human innate immune response

Nerofe induces (in-vitro) stimulatory anti cancer receptors

CD336 – specific stimulatory receptor of NK cells.
CD137- specific anti-cancer stimulatory receptor for CD8, NK cells, DC.
CD314 – specific stimulatory of activated CD8, NK cells

Effects of Nerofe on expression of stimulatory anti-cancer receptors on membrane of hPBLs (in-vitro,FACS experiments)



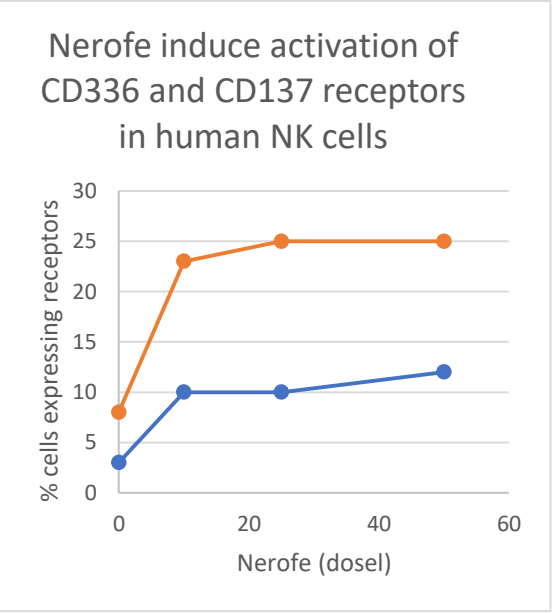
Effect of Nerofe on human innate immune response

Nerofe directly induces (in-vitro) stimulatory anti cancer receptors

CD336 – specific stimulatory receptor of NK cells.
CD137- specific anti-cancer stimulatory receptor expressed on stimulated NK cells.

Nerofe directly induces expression of stimulatory anti-cancer receptors on membrane of human NK cells (in-vitro,FACS experiments)

Human NK cells , were purchase from Lonza and were exposed to different doses of Nerofe. Expression of **CD336** and **CD137** was tested using FACS analysis.



Nerofe – Phase 1

Phase 1 - Details

Rationale

- Nerofe is aimed at curing AML/MDS.
- Upon FDA recommendation, in order to facilitate Nerofe's clinical development, we did Phase 1-(dose confirmation stage) with "all comers" solid tumor patients without limitations of number of lines of treatment and of types of cancer diseases.

Patient Population (total of 20 advanced cancer patients)

- 15 deteriorating patients entered the trial while having been treated for various types of cancer with at least 4 lines of treatment.
- 4 patients suffered from pancreatic cancer and had received 2 lines of treatment prior to entering the trial.
- 1 patient had not been pre-treated.

Phase 1 – Additional Observations

Progression Free Survival (PFS) was observed in 7 out of 20 patients (during months 3.5, 4, 6 and 12.)

Responses – we had one patient with complete pathological response. The patient with spinal cord neoplasm was not able to walk and suffered from pain that 5 "pain killers" were not able to overcome. 5 month after starting of treatment the patient walked freely with no pain as she was treated with only one anti-pain medication. After 12 month of treatment the patient went through surgery to take out what is left from tumor and pathology examination revealed the tumor became benign. Another patient with ovarian cancer (now under treatment) and spread metastasis in peritoneum came into trial with fair amount of water in peritoneum and CA-125 level of 130, after three month of treatment the tumor was stable, amount of water drastically decreased and marker decreased to 100.(patient is under treatment)

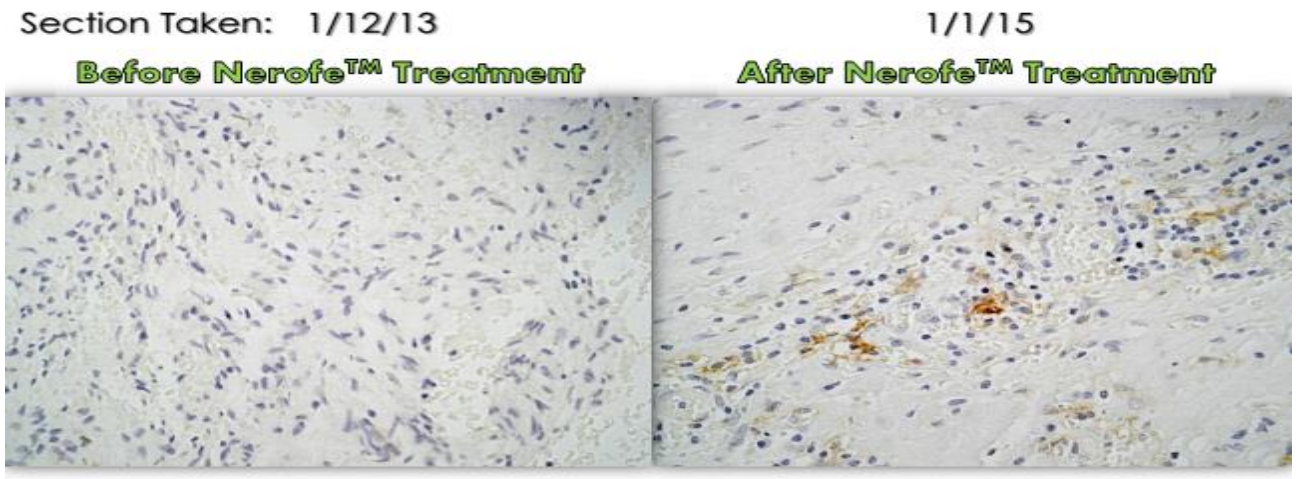
A strong multiple-factor anti-angiogenesis effect was observed in all patients of two cohorts, showing orders of magnitude decrease of the following plasma angiogenesis factors: VEGF-A, VEGF-D, PDGF-AA, PDGF-BB, aFGF, bFGF and Angiopoietin-1.

A strong anti-proliferative effect. In 3 patients high levels of EGF were decreased to the normal levels.

An immune modulatory effect and selective biomarker to pre-assess treatment efficacy. **All patients with biopsies positively stained to ST2 receptor have demonstrated an increase in TNF-alpha, IL-2, IL-21, IL-12p70 and GM-CSF plasma levels during the treatment. Their PFS was longer than 3 months.**

Nerofe induced complete pathological response in a patient with spinal cord neoplasm

Biopsy before entering trial	Biopsy after finishing trial
More than 30% of cells are Ki67 positive	5-10% of cells are Ki67 positive
CD31 positive	CD31 negative - bleeding due to absence of blood vessels
No scar	Scar present due to immune reaction
Neoplasm	Benign

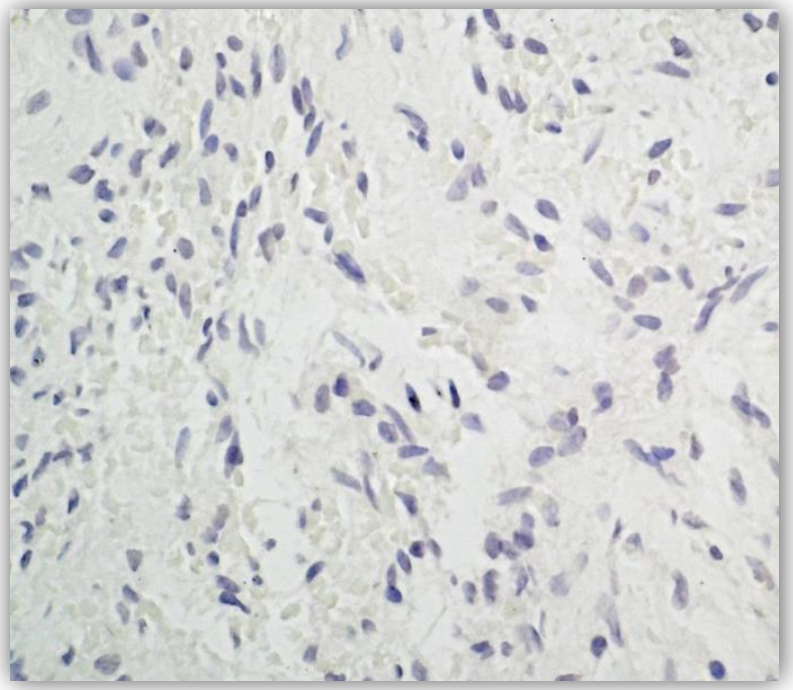


Nerofe allowed a crippled widow to stand up and walk

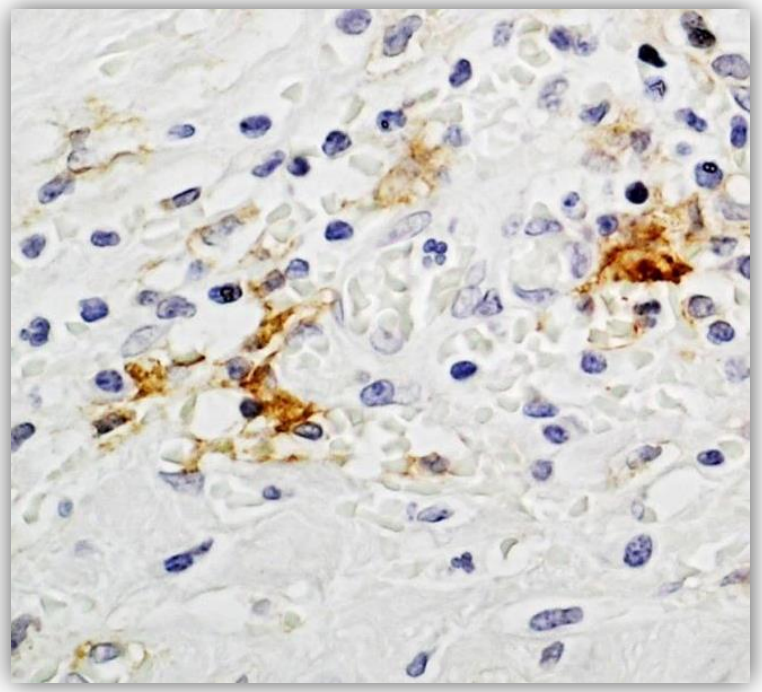
Nerofe™ -

increases immunogenicity of cancer cells in human tumor

NK cells activation in human tumor (CD11c)



Before Nerofe ™



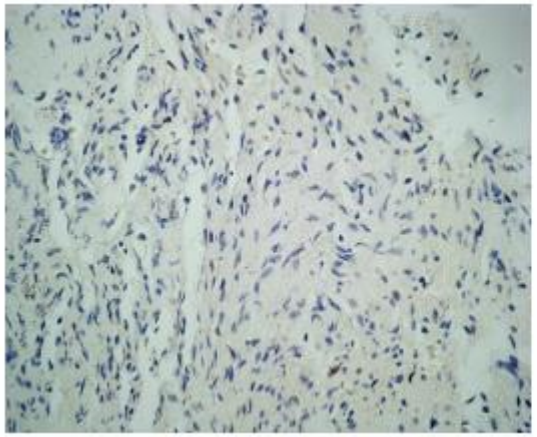
After Nerofe ™

Due to "eat me " signal on cancer cells NK cells arrives and kill the cancer cells

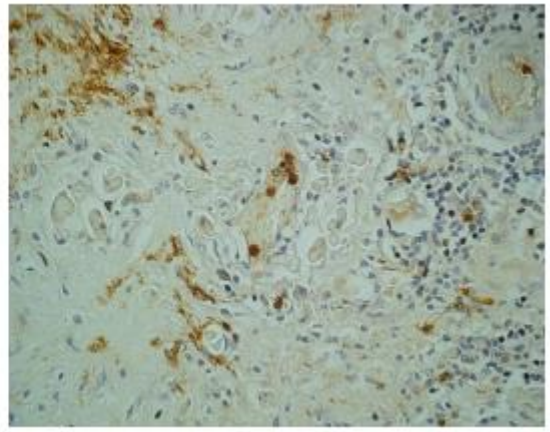
Nerofe™ -

increases immunogenicity of cancer cells in human tumor

DC cells activation in human tumor



Before Nerofe™

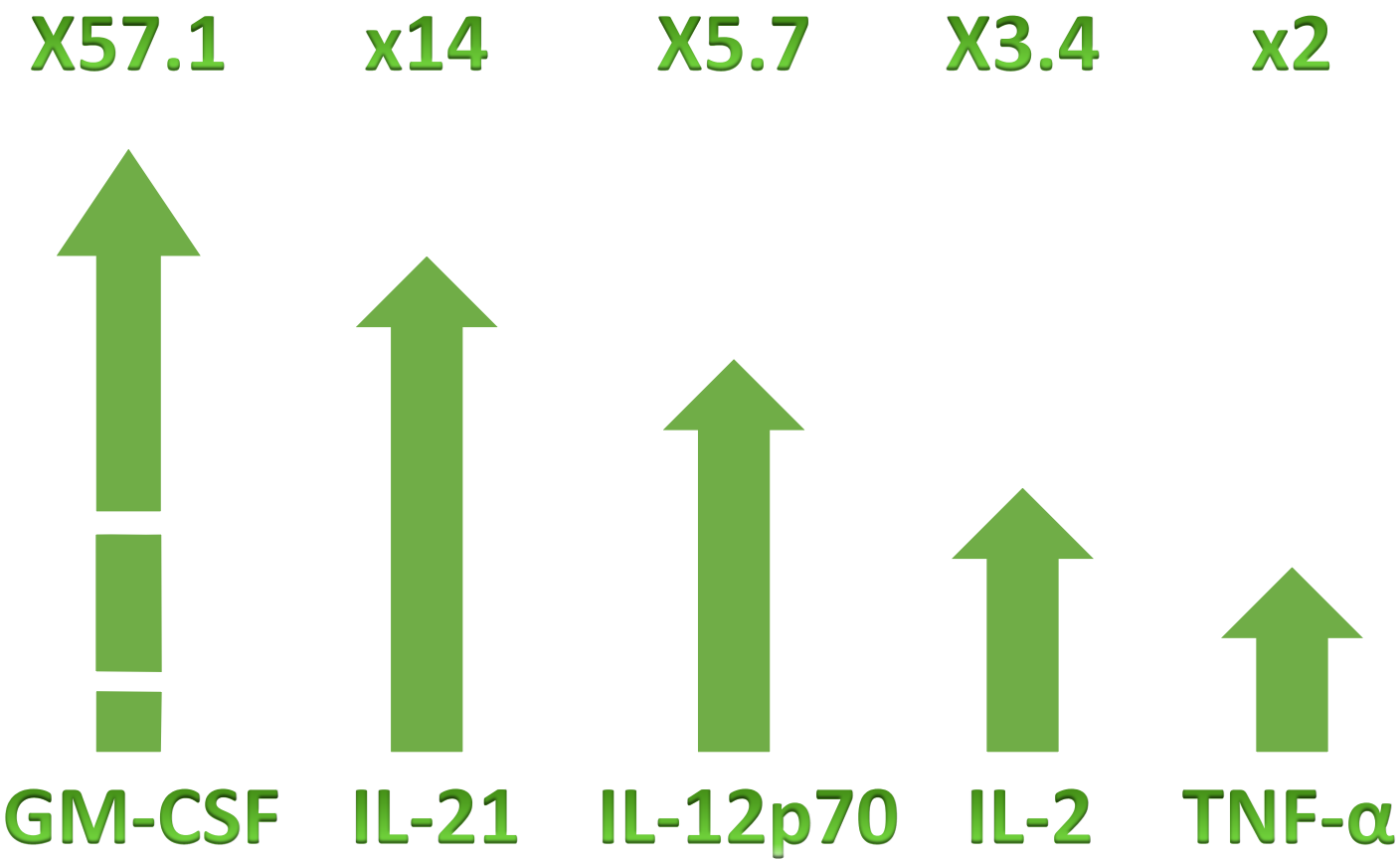


After Nerofe™

Due to "eat me signal" on cancer cells DC cells arrives and kill the cancer cells

Nerofe™ - increases anti-cancer immune response

Serum Cytokines raise in human trial (average)



Nerofe strongly induces anti-cancer immune cytokine in human patients treated with Nerofe

Nerofe conducts on novel immunotherapy concert

Nerofe

Activation of NK cells:
Increase expression of
CD336 and CD137

NK cells

Increase sensitivity to Chemo X by induction of CHOP

ST2 positive Cancer cells

Low doses of

Chemo X

Induces apoptosis in cancer cells
Increase sensitivity to NK cells :
Increase expression of DR5
and decrease expression of Flip

Effect of combination Nerofe +ChemoX on hTNBC tumors

32 nude mice were inoculated SC with 9 million hTNBC cells per mouse. When tumors exceeded volume of 40(mm^3) mice were divided randomly into 5 groups groups:

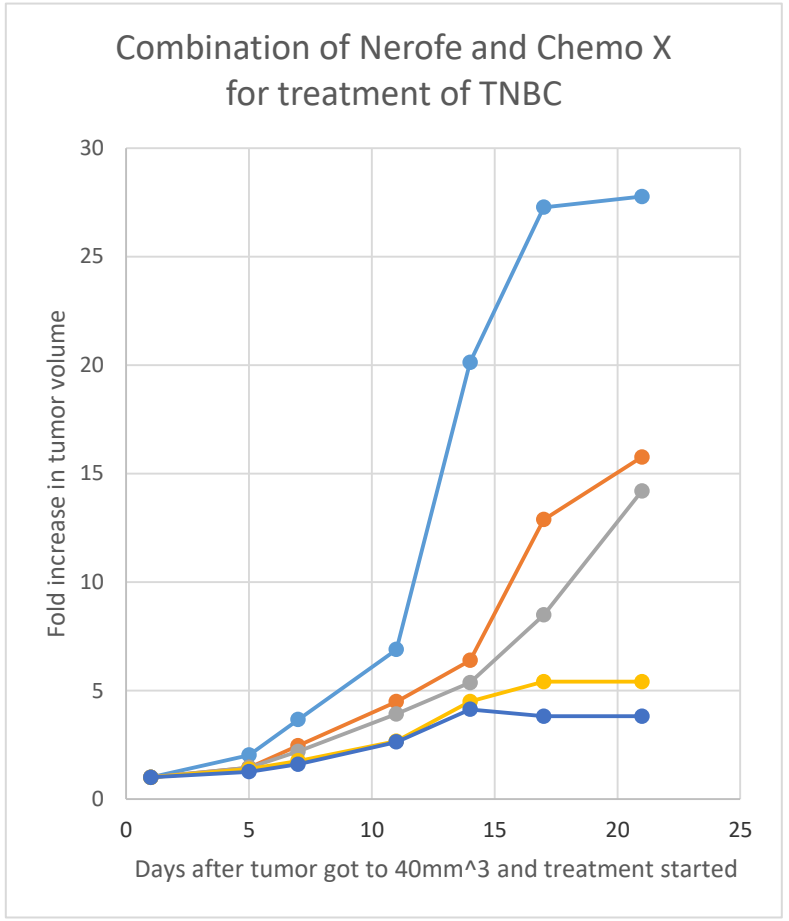
Control group (n=5) was treated with 5% mannitol

Nerofe treated group (n=5) once a week (15mg/Kg)

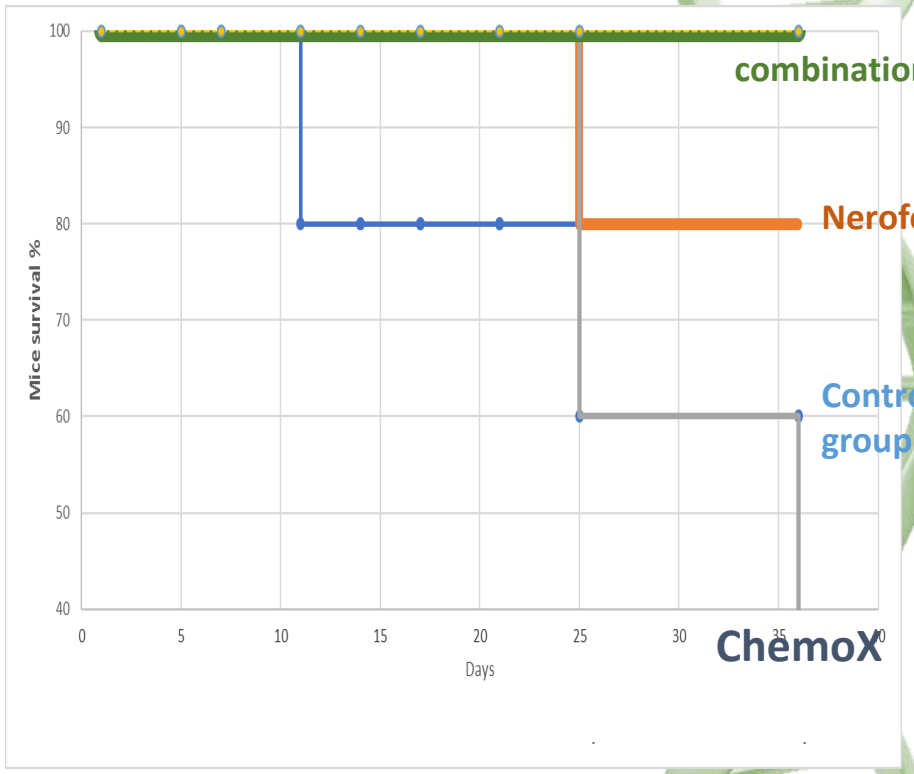
Chemo X treated group (n+5) once a week (3mg/kg)

Nerofe +ChemoX (Nerofe treatment and Dox 24hr later) (n=8)

Nerofe+ChemoX (Nerofe treatment and Dox on same day)(n=9)



Survival rate of mice inoculated with hTNBC tumor and treated with Nerofe and Chemo X



Effect of combination Nerofe +Chemo X on mice melanoma tumors

38 nude mice were inoculated SC with 4 million B16 cells per mouse. When tumors exceeded volume of 40(mm^3) mice were divided randomly into 5 groups groups:

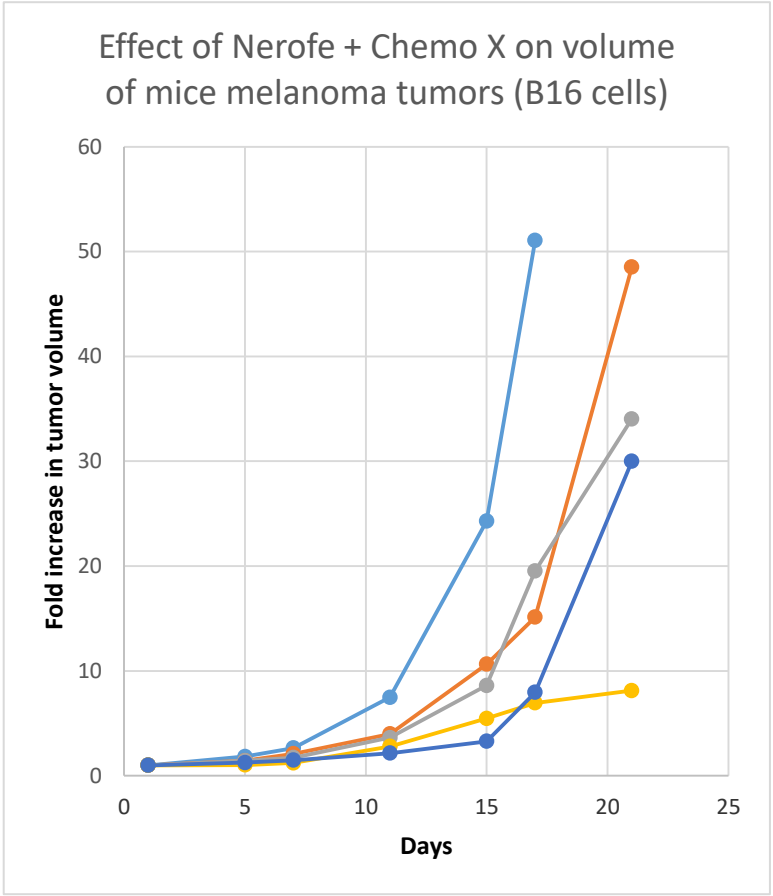
Control group (n=6) was treated with 5% mannitol

Nerofe treated group (n=6) once a week (15mg/Kg)

Chemo X treated group (n=6) once a week (3mg/kg)

Nerofe +Chemo X (Nerofe treatment and Dox 24hr later) (n=10)

Nerofe+Chemo X (Nerofe treatment and Dox on same day)(n=10)



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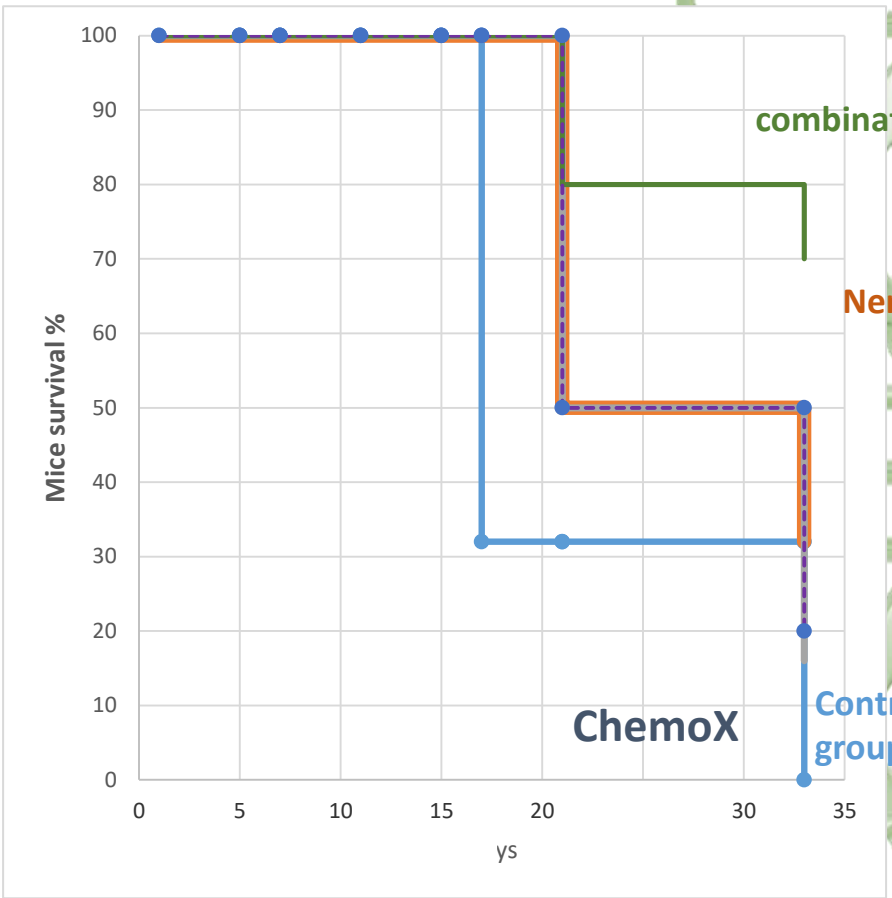
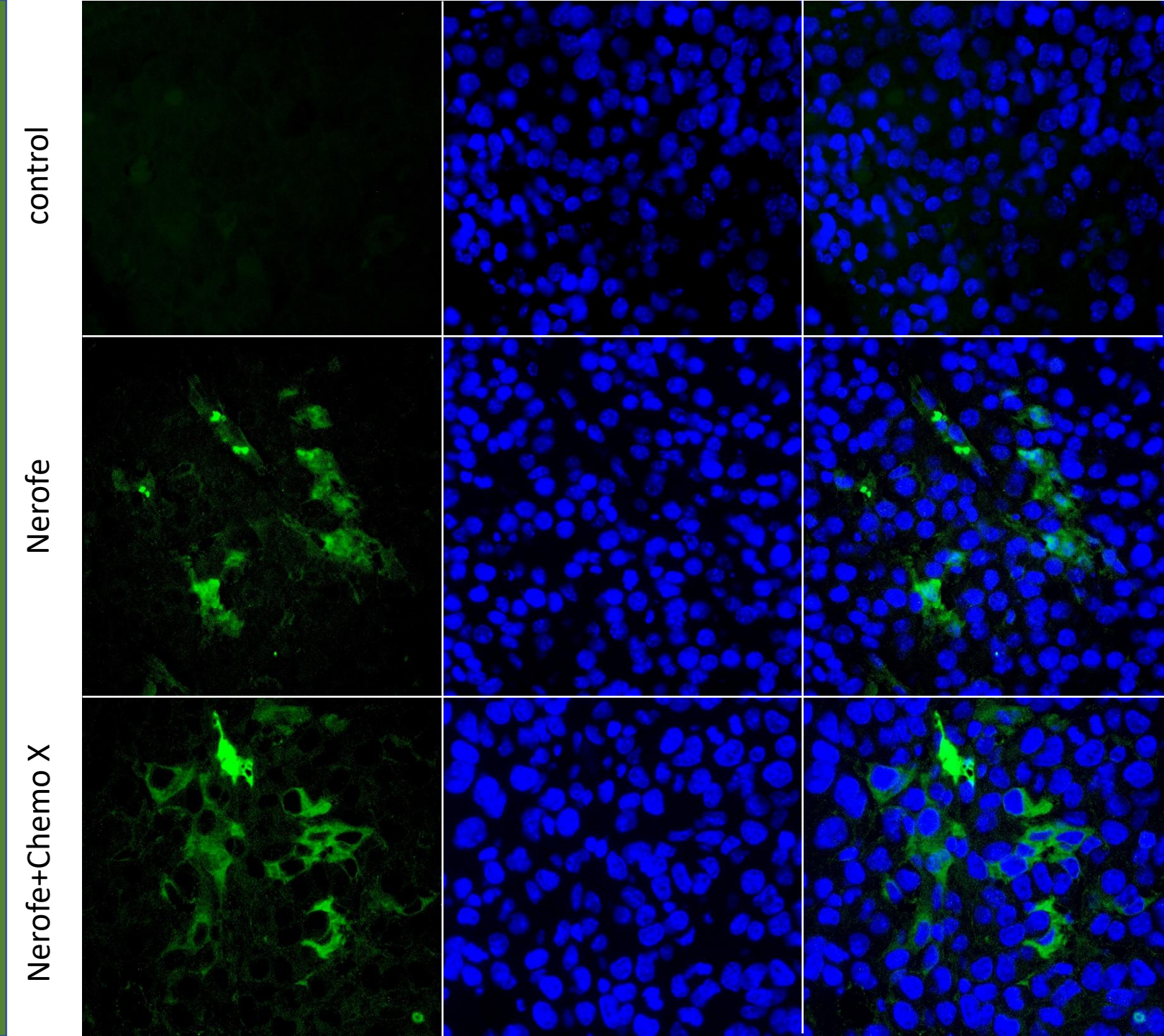
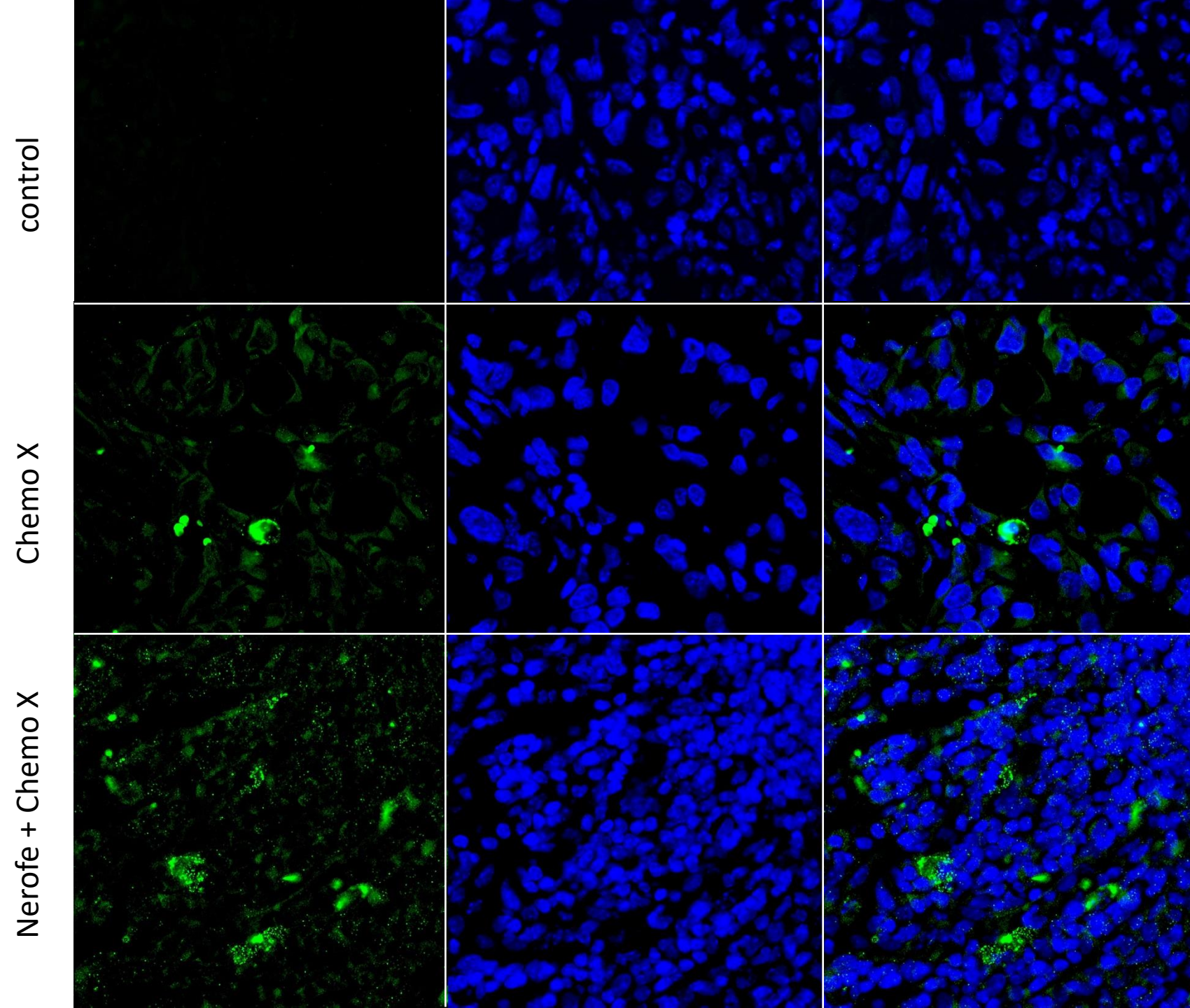


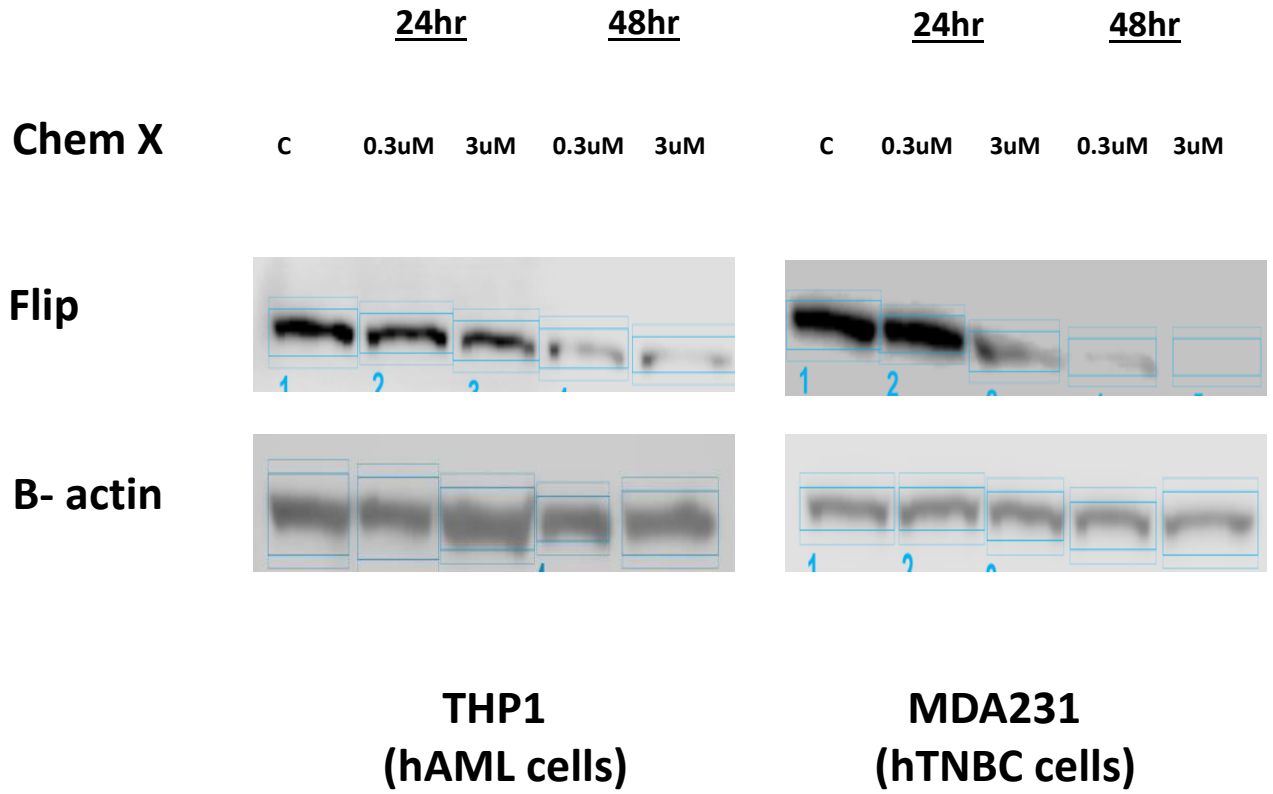
Nerofe induces CHOP in human cancer tumor and make them sensitive to Chemo X



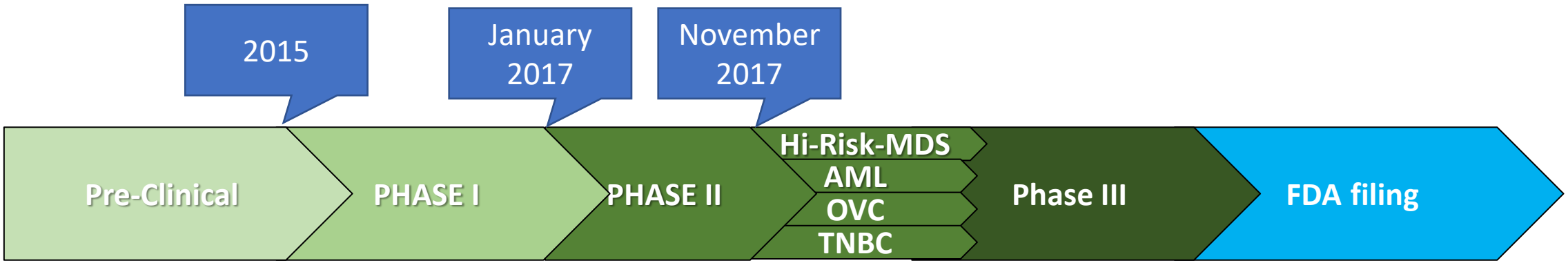
ChemoX induces DR5 in human cancer tumor and make them sensitive to NK cells



ChemoX induces Flip degradation in human cancer tumor and make them sensitive to NK cells



Clinical plan



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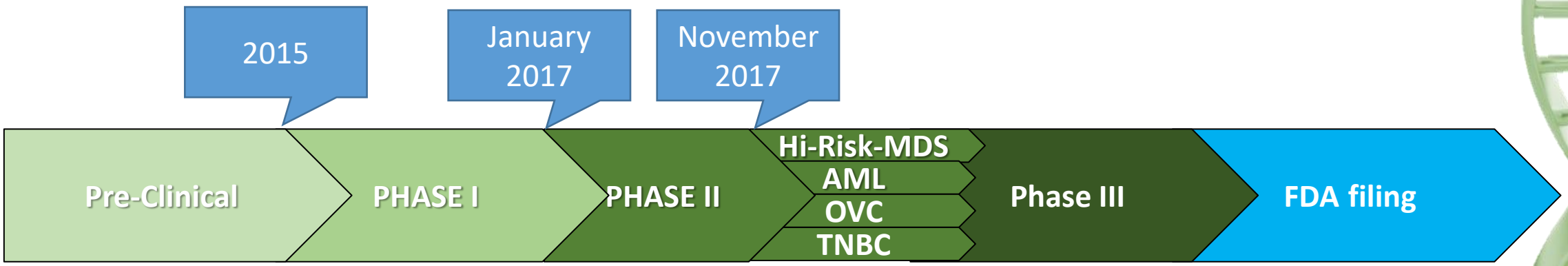
Clinical plan



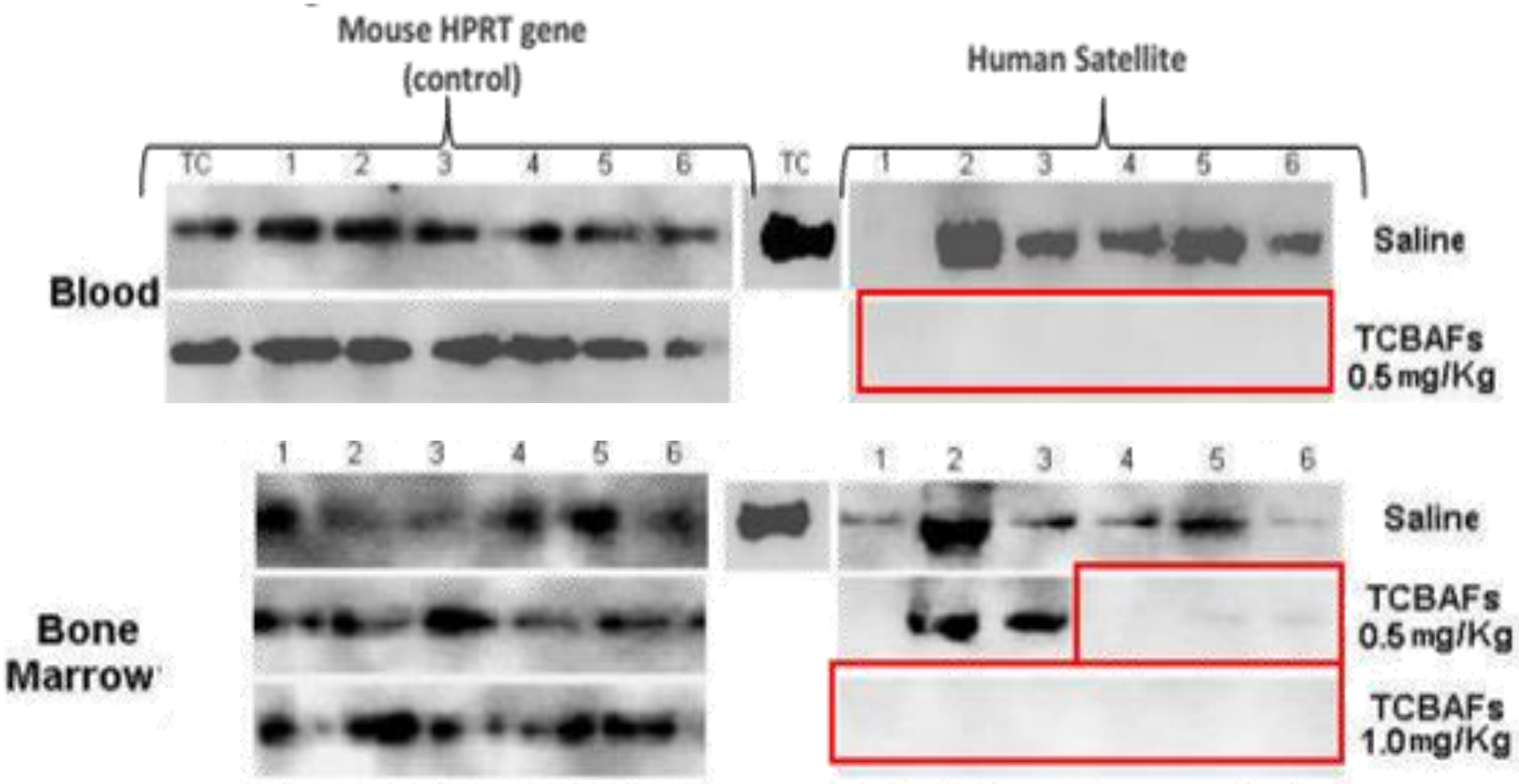
Clinical Plan

Based on in-vitro, in-vivo and phase 1 results we decided to on the following clinical plan

- Phase 2a – AML/ Hi Risk MDS (in Israel and Europa due to low number of patients in Israel)
- Phase 2a – TNBC / Ovarian Cancer (in Israel)

Nerofe™ - an efficient drug candidate for AML

Currently the only known drug able to totally eliminate cancer cells from bone marrow



Mice were injected with human AML cells (U937 cell line), once cells penetrated bone marrow mice started IP administration treatment with different doses of Nerofe vs. saline (control group). Detection of human cells in blood and bone marrow was done with RT-PCR looking at human satellite(40 cycles). We can see clearly that Nerofe caused complete disapprence of human AML cells from bone marrow and blood in a dose depended manner.
Same results were obtained with ML2 cell line