2nd International Electronic Conference on Medicinal Chemistry

1-30 November 2016
chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Indeno[1,2-*b*]indole Inhibitors of Human Protein Kinase CK2 and Their Impact on Different Tumor Cell Lines

Dagmar Aichele[1,*], Marc Le Borgne[2], Joachim Jose[1]

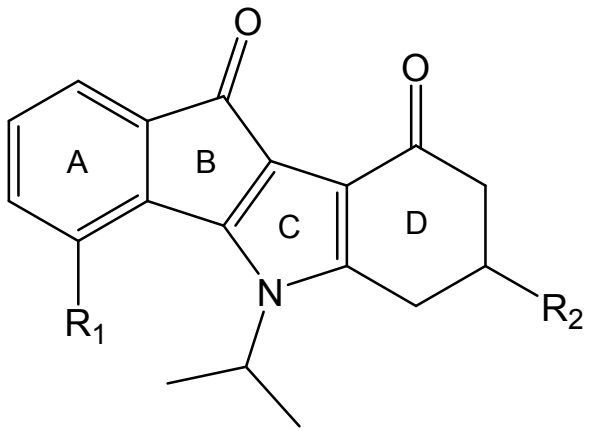
[1] Institute of Pharmaceutical and Medicinal Chemistry, Westfälische Wilhelms-Universität, PharmaCampus, Corrensstraße 48, 48149 Münster, Germany;
[2] ISPB-Faculté de Pharmacie, Université Claude Bernard Lyon 1, EA 4446 Bioactive Molecules and Medicinal Chemistry, 8 avenue Rockefeller, 69373 Lyon cedex 08, France.

* Corresponding author: dagmar.Aichele@uni-muenster.de

Westfälische Wilhelms-Universität Münster

PharmaCampus
Institut für Pharmazeutische und Medizinische Chemie

Indeno[1,2-*b*]indole Inhibitors of Human Protein Kinase CK2 and Their Impact on Different Tumor Cell Lines



Structure of indeno[1,2-b]indole-9,10-dione derivatives tested on tumor cell proliferation

Abstract: Increased protein kinase CK2 activity is involved in many human diseases such as cancer (1). In consequence CK2 is an emerging major target for drug design. Several indeno[1,2-b]indole-9,10-dione derivatives containing N^5-isopropyl substitutions on the C-Ring were synthesized and have been reported as potent ATP-competitive CK2 inhibitors (2,3).

Here we report on the evaluation of these inhibitors, containing different substituents in the A- and D-rings, for their effects on various tumor cell lines: breast cancer cells MCF-7, lung carcinoma cells A427 and epidermal cancer cell line A431. The most potent CK2 inhibitor contains an O-prenyl residue R_1 and exhibits an IC_{50} value of 0,025 µM. Treatment of MCF-7 cells with 20 µM of that compound for 24 h results in a reduction of the total cell number by 90%. Most of the remaining cells exhibited apoptotic morphology and showed nearly none proliferating activity. In contrast treatment of A431 cells and A427 cells caused only a moderate decrease of cell proliferation by 30% for all tested compounds.

This study shows that potent CK2 inhibitors as tested can exhibit distinct effects on different tumor cell lines. Compound containing an O-prenyl residue R_1 appears to be an antiproliferating agent with high activity toward MCF7 cells.

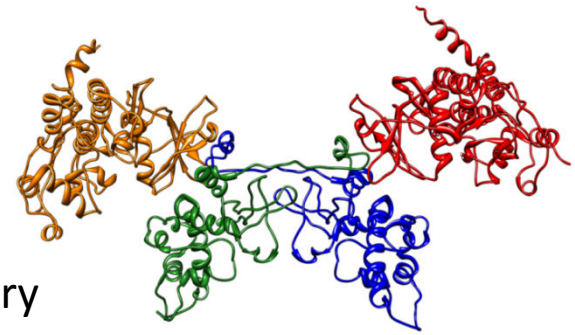
Keywords: CK2, CK2 inhibitors, Indeno[1,2-b]indole-9,10-dione, cell proliferation

References: (1) Guisiano, S. et al: *Eur. J. Cancer* **2011**, 47, 792-801 (2) Alchab, F. et al : *Pharmaceuticals* **2015**, 8, 279-302
(3) Gozzi, et al: *J. Med. Chem* **2015,** 58, 265-277

Introduction

Protein kinase CK2

- pleiotropic and ubiquitous enzyme
- constitutively active serine/threonine kinase
- heterotetrameric enzyme



- key role in several cellular processes (DNA repair, cell growth, cell cycle)
- composed of two catalytic (α and/or α`) and two regulatory subunits β
- is upregulated in cancers
- major role in cell death
- connected to many human diseases neurodegenartive, inflammatory diseases, cancer)

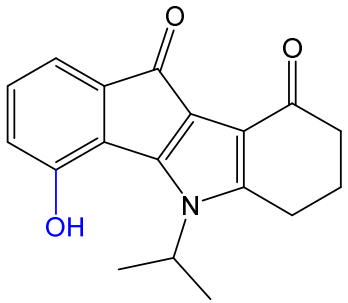
➡ **important target for cancer therapy**

Introduction

CK2 inhibitors

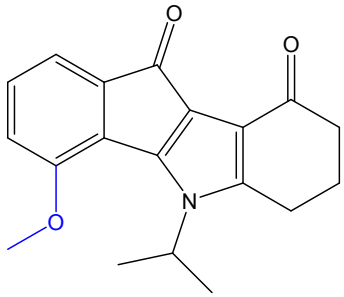
- Development of CK2 inhibitors represents a promising strategy in cancer therapy
- Most of the described CK2 inhibitors are ATP-competive.
- Among this the highly selective drug CX-4945 is the first CK2 inhibitor in clinical trials.
- A series of indeno[1,2-*b*]indole-9,10-dione derivatives was synthesized as CK2 inhibitors.
- The most potent derivatives contained a N^5-isopropyl residue on the C-ring.

Structure and IC_{50} values of tested CK2 inhibitors



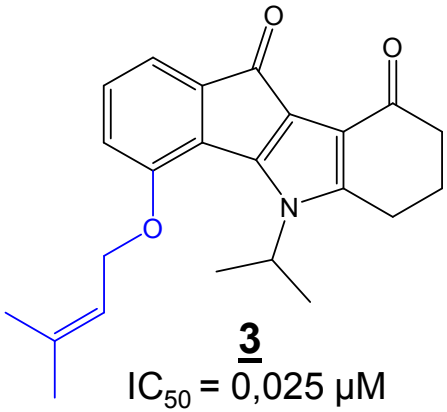
1

IC_{50} = 0,28 μM



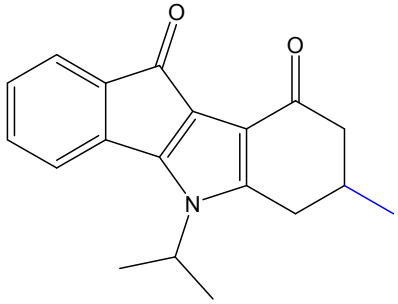
2

IC_{50} = 0,071 μM



3

IC_{50} = 0,025 μM



4

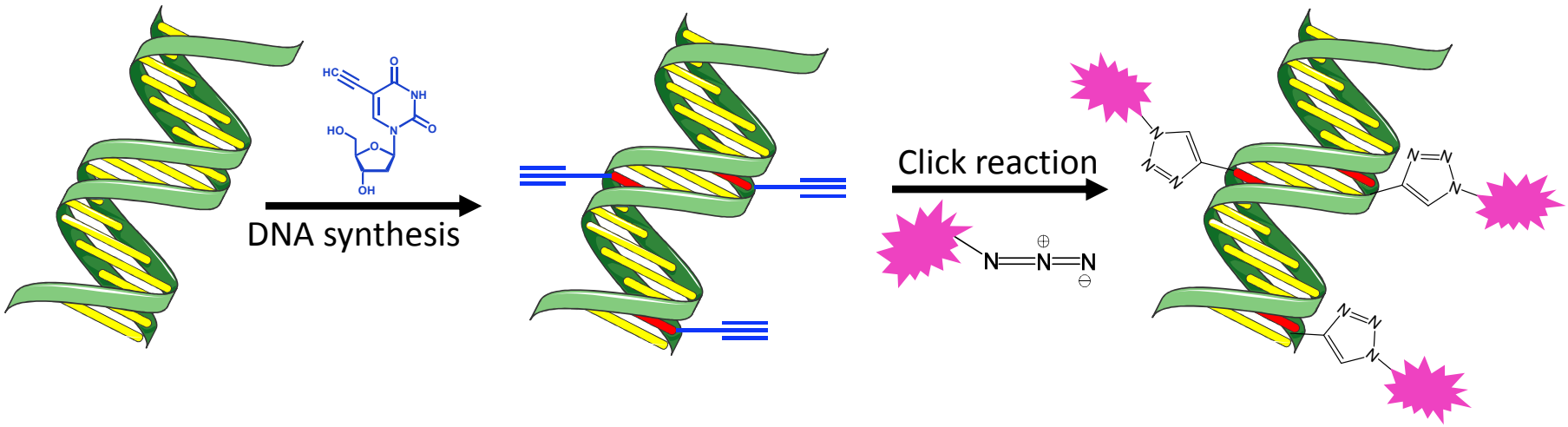
IC_{50} = 0,17 μM

Indeno[1,2-b]indole-9,10-dione derivatives containing N^5-isopropyl have been reported as potent CK2 inhibitors. CK2 inhibitors evaluated in this study revealed IC_{50} values in the submicromolar range.

Testing of the inhibitors of the human CK2 was performed by capillary electrophoresis based CK2 activity assay.

Gratz et al., *Electrophoresis*, **2010**, 31, 634-640.

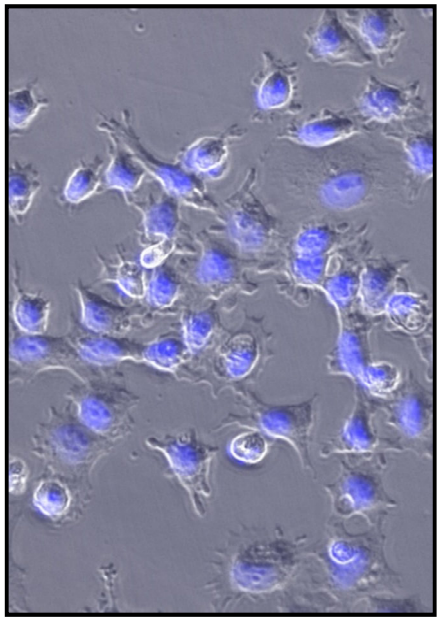
Detection of cell proliferation by EdU assay



EdU (5-ethynyl-2`-doxyuridine) is a nucleoside analog to thymidine and is incorporated during DNA synthesis. For detection EdU assay utilizes click chemistry. The 5-TAMRA-PEG3-azide fluorophore is coupled to incorporate EdU by click reaction. Cellular fluorescence of proliferating cells can be monitored with a fluorescence microscope.

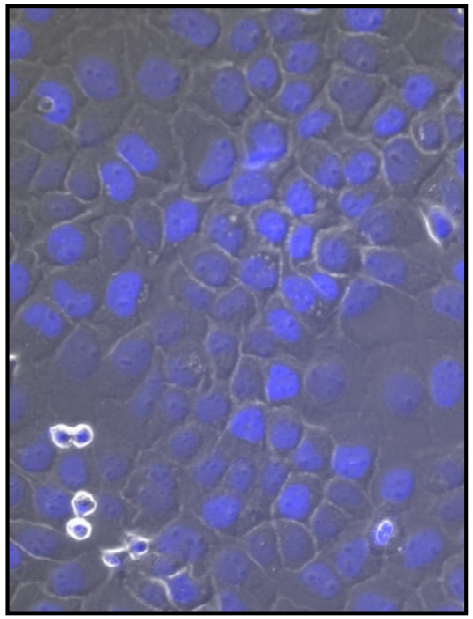
Cell lines used for testing CK2 inhibitors

A427 cells



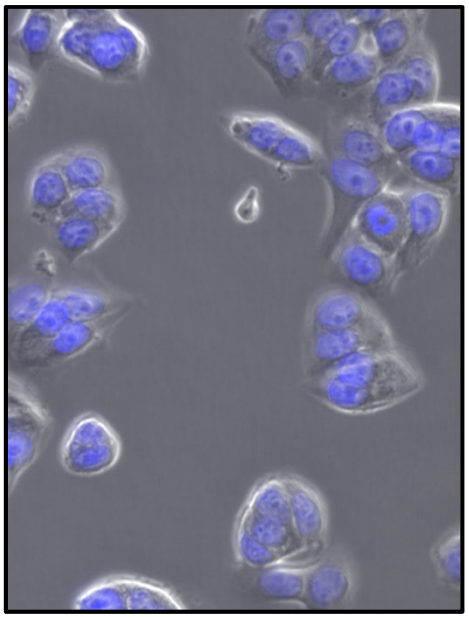
- adherent
- lung carcinoma
- epithelial
- 52 years, male

A431 cells



- adherent
- epidermoid carcinoma
- epithelial like
- 85 years, female

MCF-7 cells

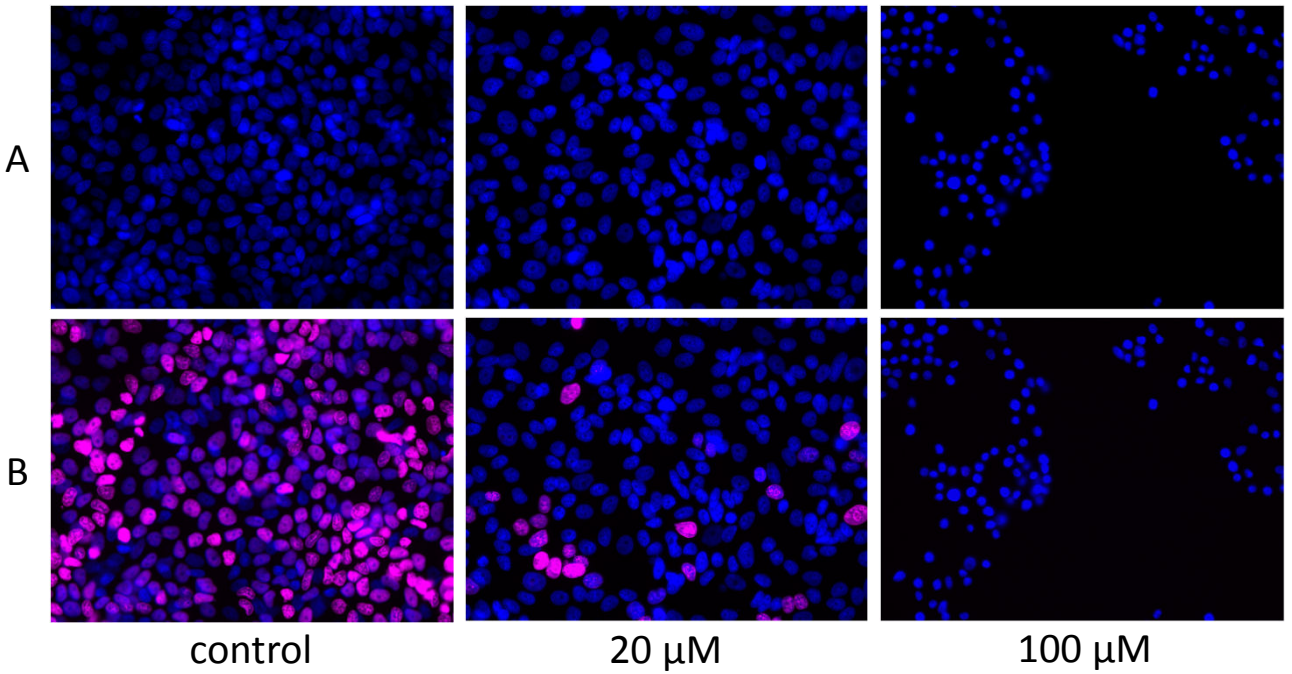


- adherent
- breast adenocarcinoma
- epithelial like
- 69 years, female

Cell nuclei were stained using fluorescent dye Hoechst- 33342. Phase-contrast images and fluorescence images were overlayed.

Results and discussion

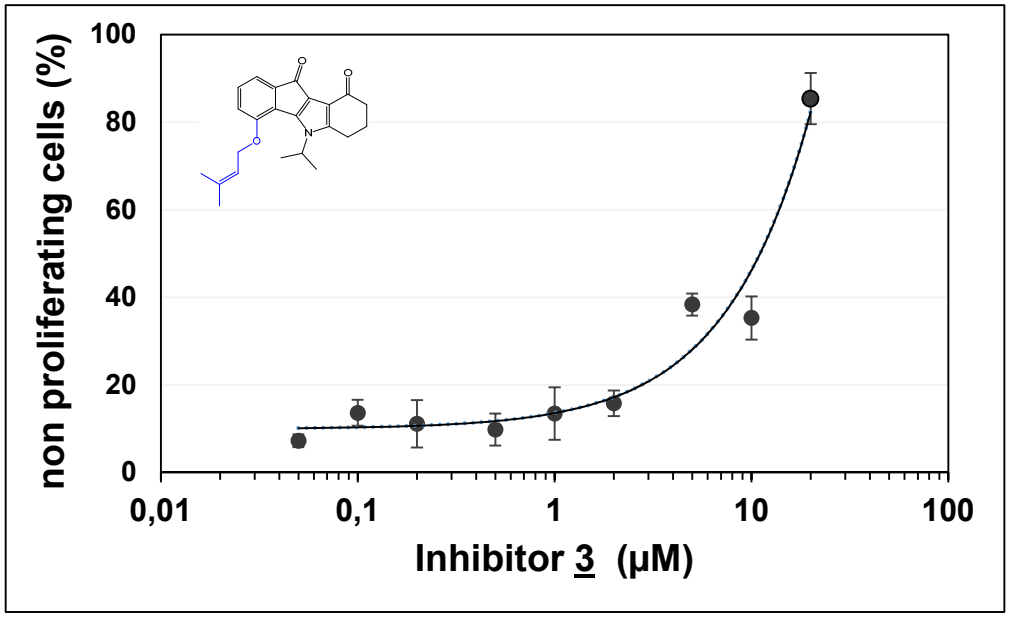
Inhibition of MCF-7 cell proliferation by CK2 inhibitor 3



MCF-7 cells were treated with 1% DMSO as control and CK2 inhibitor **3** (20 µM and 100 µM) for 24 h. Cell nuclei of all cells were stained with Hoechst 33342. These cells are emitting a blue fluorescence **(A)**. Only proliferating cells are emitting an additional violet fluorescence due to staining with 5-TAMRA-PEG3-azide fluorophore **(B)**.
Incubation of MCF-7 cells with 20 µM inhibitor **3** for 24 h resulted in a dramatic reduction of cell proliferation. When concentration is increased to 100 µM, cell proliferation were completely inhibited after 24h exposure and the remaining cells showed characteristic features of apoptosis such as condensation of chromatin.

Results and discussion

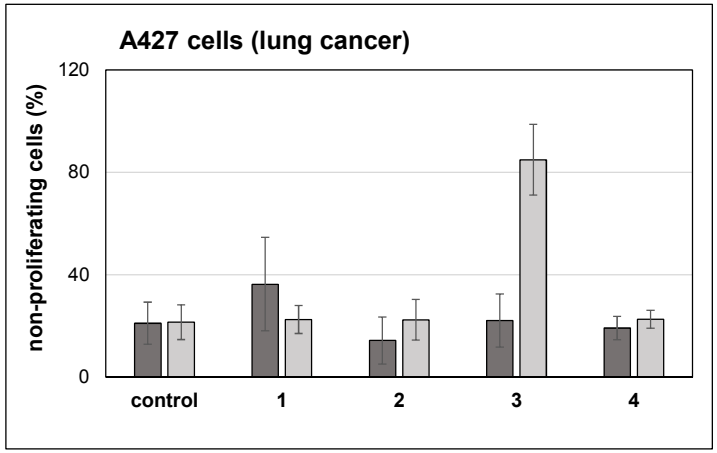
Dose dependent inhibition of cell proliferation

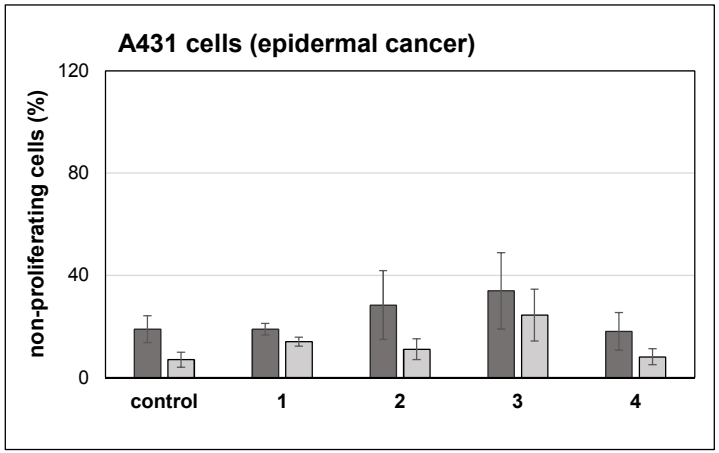


MCF-cells were treated with CK2 inhibitor **3** at different concentrations for 24 h. The total cell number (staining with Hoechst 33342) and the number of proliferating cells (Tamra labeled cells) were counted. The results are expressed as a percentage of non-proliferating cells versus total cell number.
As the diagram shows, 50% of the cells were not able to synthesized DNA, when they are treated with approximately 15 µM of that inhibitor.

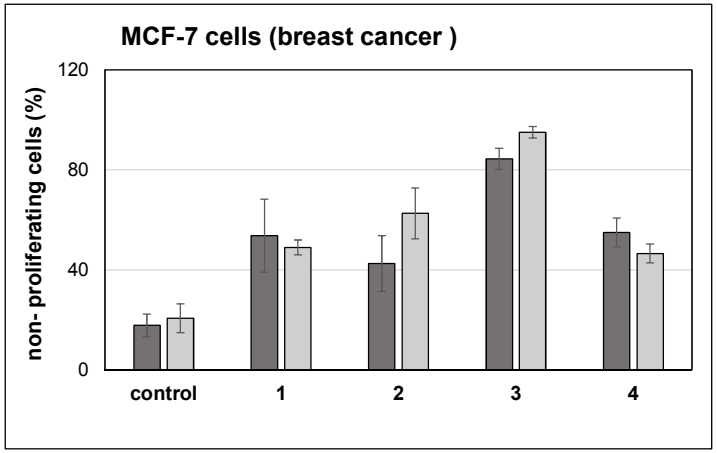
Results and discussion

Influence of CK2 inhibitors on cell proliferation of various tumor cells





24 h
48 h



Cells were treated with 20 µM CK2 inhibitor or with 1% DMSO as control. The total cell number and the number of non-proliferating cells were determined. The results are shown as a percentage of non-proliferating cells versus total cell number.

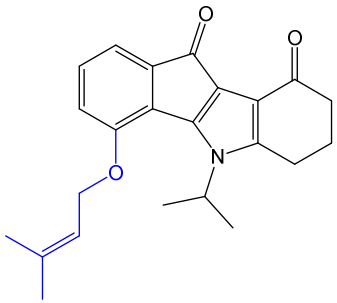
All examined inhibitors caused reduction of cell proliferation of MCF-7 cells, whereas cell proliferation of A431 cells are moderatly affected. Number of non-proliferating cells of lung cancer cells is increased to 85% only by treatment with inhibitor **3** for 48 h. Coumpounds **1**, **2**, and **4** didn't show any effect to cell proliferation of A427.

Conclusions

Our findings show that evaluated potent CK2 inhibitors could decrease the cell proliferation of tumor cell lines. Best effects were observed for MCF-7. Cell proliferation was reduced by more than 80% after 24 h, whereas proliferation of A427 was affected only after 48 h. A431 cells were influenced by compound **3** only in a moderate range.

Compound **3** was proved to be a strong antiproliferating agent.

EdU-click reaction allows to study the effects of these inhibitors on cell cycle in further experiments.



Structure of compound **3**

Acknowledgements

Many thanks to I. Finger and J. Pataillot for technical assistance.