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In-vitro Evaluation of IND126, a KRASG12C Inhibitor in Combination with Inhibitors of EGFR,Cdk 4/6 and PI3Kalpha

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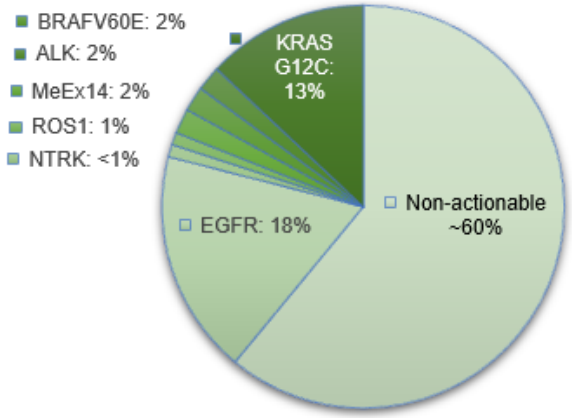
Background



Lung Cancer (NSCLC)

Leading cause of deaths worldwide

- 1.76 mln deaths/Yr[1]
- 2.0 mln New cases/Yr
- 5-Yr survival rate of 18%

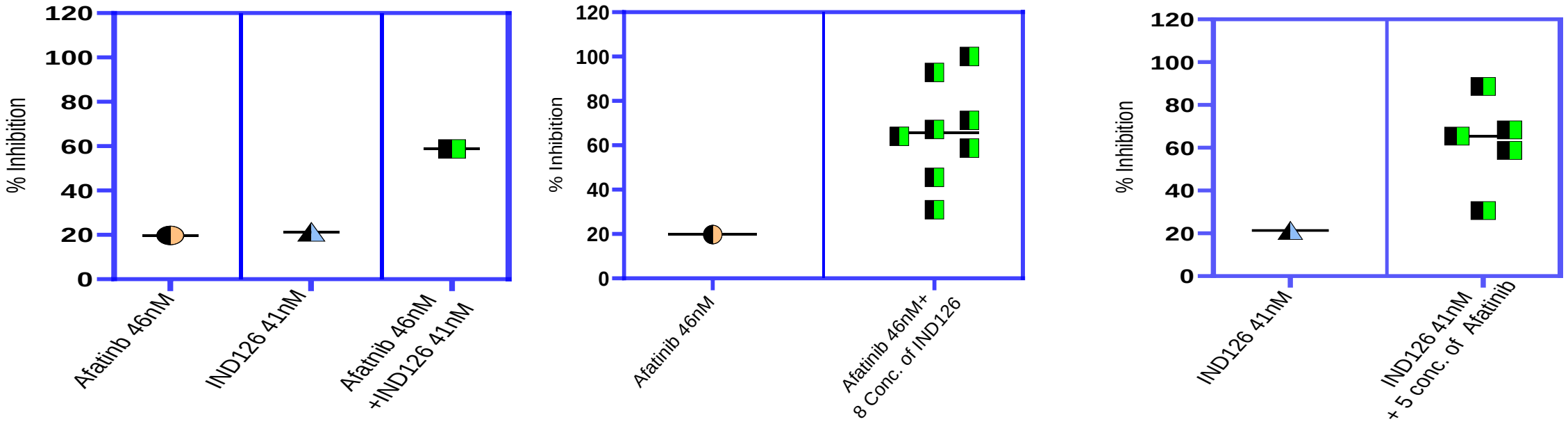
KRASG12C in NSCLC, CRC & Others

KRASG12C mutation seen in

- 13% of NSCLC[2]
- 4% of Colorectal[3]
- 2 % of other solid cancer

- ❑ Treatment with KRASG12C inhibitors as single agent (e.g.Sotorasib and Adagrasib) have shown to have a transient inhibitory effect on overall KRAS signalling, because such initial oncoprotein signalling inhibition is accompani ed by re-accumulation of active KRAS and/or reactivation of alternative pathw ays including MAPK pathway such as RAF and/or ERK.
- ❑ Emerging data also show conferring resistance to these inhibitors and supports the need for development of additional KRASG12C inhibitors.[4]
- ❑ Current Inhibitors have shown marginal or no clinical benefit in Colorectal cancer patients harbouring KRASG12C mutation.
- ❑ Development of effective combination therapy regimens is required to expand the current label offering as well fully combat resistance.
- ❑ One approach towards overcoming the acquired resistance towards KRASG12C inhibitors, is combining them with other inhibitors involved in Ras /Raf/MEK/TKI pathway.
- ❑ IND126 is a novel, potent and highly selective inhibitor of KRASG12C and is currently being pursued for IND enabling studies.
- ❑ Here we report preclinical data for combination of IND126, with Afatinib (an approved EGFR inhibitor), Palbociclib (CDk4/6 inhibitor) and Alpelisib (PI3kα inhibitor) in KRASG12C mutated cell line.

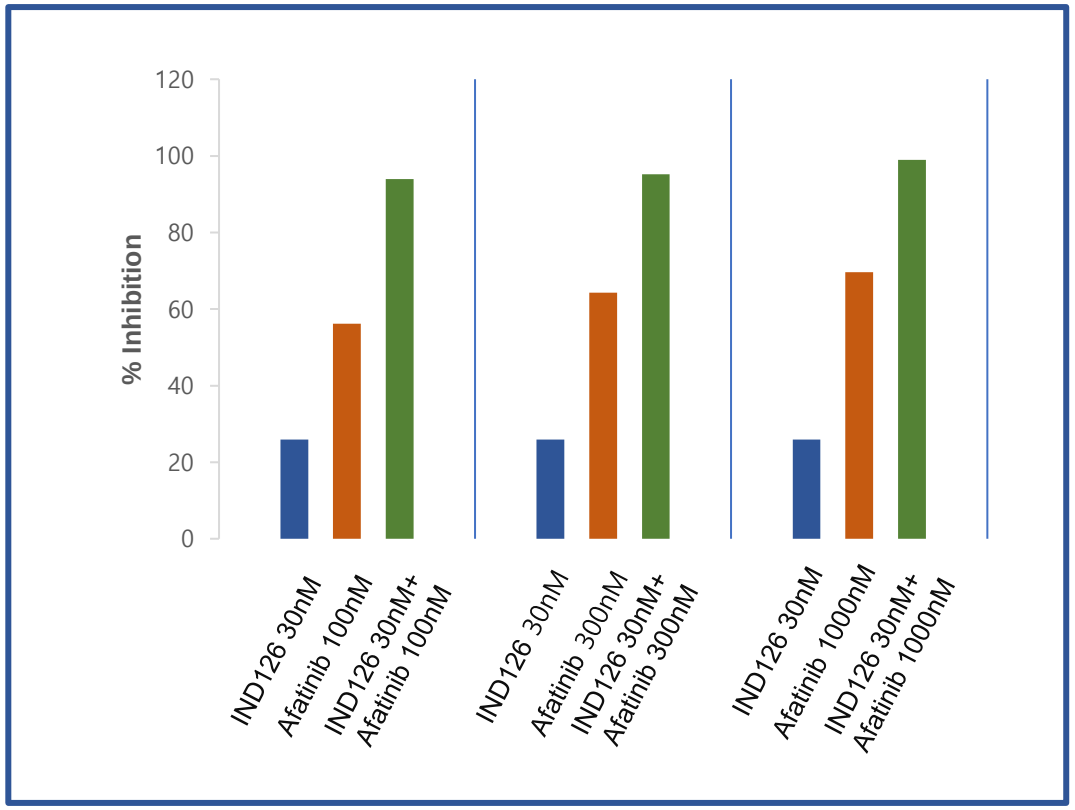
KRASG12C inhibitor, IND126 in combination with Afatinib demonstrates synergistic effect on cell viability

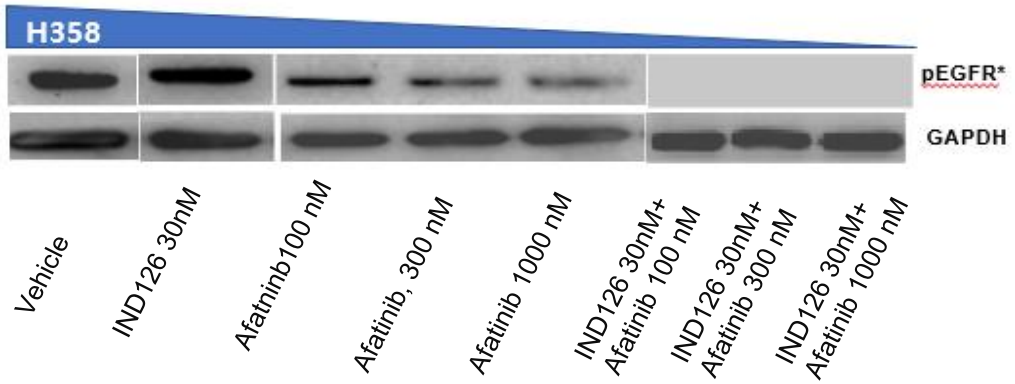


- ❑ **>50 % Inhibition observed with combination of 46 nM of Afatinib and 41 nM of IND126**
- ❑ **Combination Data analysis resulted in Composite Synergy Score of 10+^**

Cell viability assay (MTT) was performed in H358 cell line using a combination drug matrix (40 point) of IND126 (8 conc.) and Afatinib (5 conc.). Data was normalized and percent inhibition was calculated with reference to control. Conc. of IND126 used were 10,3.3, 1.1,0.37, 0.123, 0.041, 0.013 & 0.0045 µM and Afatinib ,10, 1.67, 0.278, 0.046, 0.007 µM in this study.
^ Data analysis performed using Synergy Finder

KRASG12C inhibitor, IND126 significantly potentiates pEGFR inhibitory activity of Afatinib

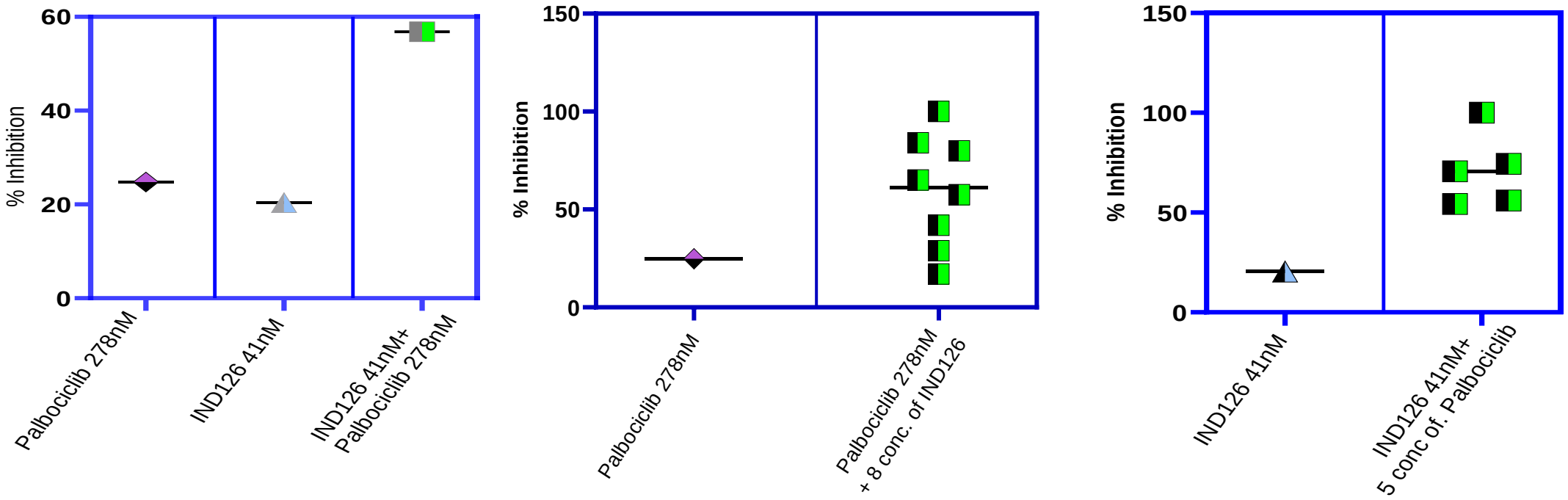




- ❑ 30 nM of IND126 synergistically potentiated pEGFR inhibition
- ❑ ~2-fold increase in activity of Afatinib upon addition of 30 nM of IND126

Western Blot Assay to evaluate modulation of pEGFR (Y1068*) in NCI-H358 cells treated with combination drug matrix (4 points) of IND126 (1 conc) and Afatinib (3 conc) at 6 hr. time point.

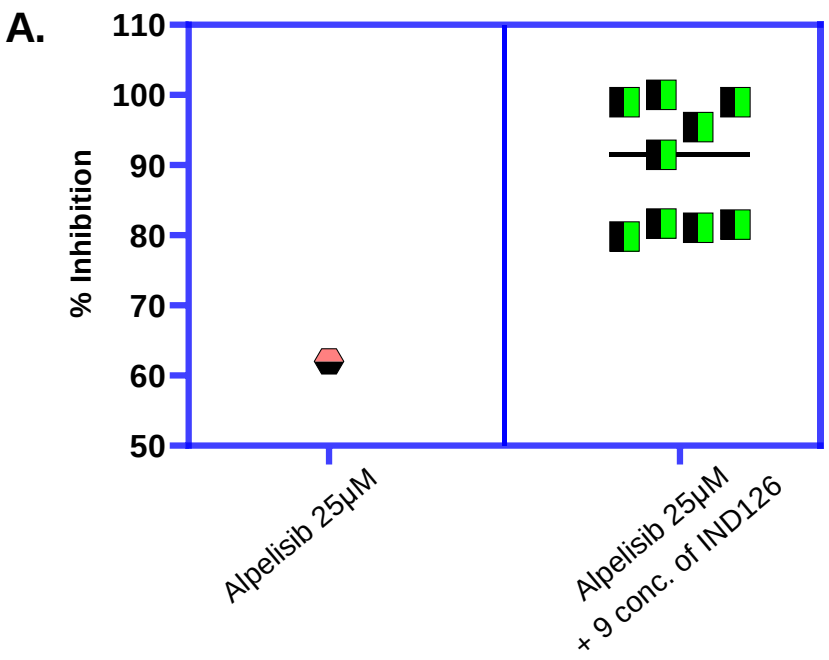
KRASG12C inhibitor, IND126 in combination with Palbociclib demonstrates synergistic effect on cell viability

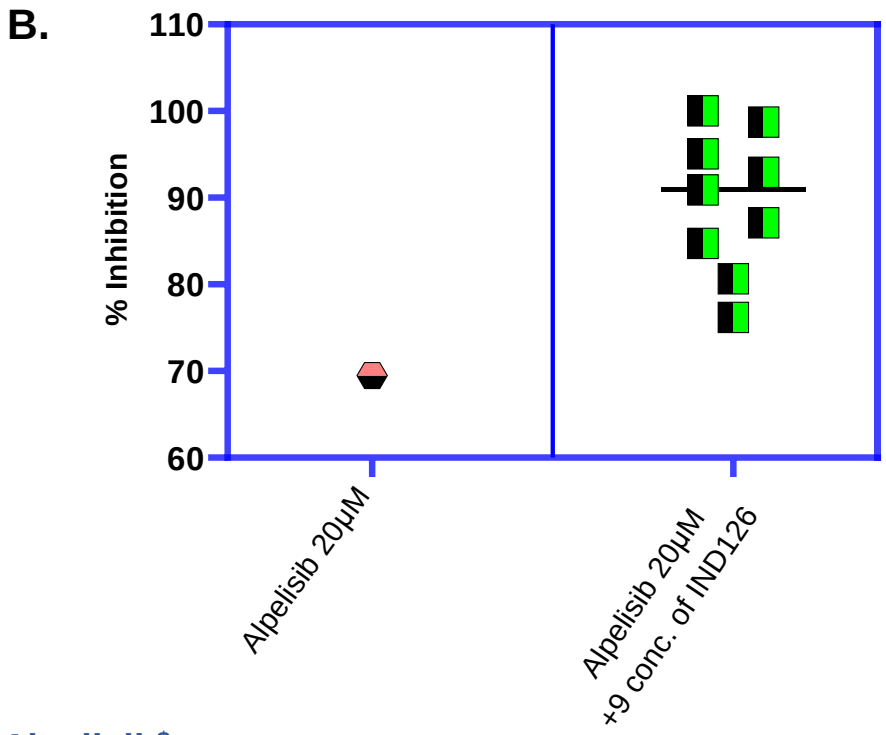


- ❑ **>50 % Inhibition observed with combination of 278 nM of Palbociclib and 41 nM of IND126**
- ❑ **Combination Data analysis resulted in Composite Synergy Score of 5+^**

Cell viability assay (MTT) was performed in MIAPaCa-2 cell line using a combination drug matrix (40 point) of IND126 (8 conc) and Palbociclib (5 conc). Data was normalized and percent inhibition was calculated with refence to control. Conc. of IND126 used were 3.3, 1.1,0.37, 0.123, 0.041, 0.013 , 0.0045 & 0.0015 µM and Palbociclib , 10, 1.6, 0.278, 0.046, 0.007 µM in this study.
^ Data analysis performed using Synergy Finder
MIAPaCa-2 , cell line was chosen due to insensitivity of Palbociclib in H358 Cell line.

Cell viability data of combination of IND126 with Alpelisib in KRASG12C mutant cell lines





- ❑ **Moderate cell Inhibition observed in combination of Alpelisib[$]**
- ❑ **Combination Data analysis resulted in low to negative Composite Synergy Score[^$]**

Cell viability assay (MTT) was performed to study combination effect of IND126 (9 conc) with (A) Alpelisib (25 µM) in H358 and (B) Alpelisib (20 µM) in MIAPaCa-2 cell lines. Data was normalized and percent inhibition was calculated with reference to control. IND126 conc. used were 10, 3.3, 1.1, 0.37, 0.123, 0.041, 0.013 , 0.0045 & 0.0015 µM;

^ Data analysis performed using Synergy Finder

$ Alpelisib have been reported to be insensitive to both MIAPaCa-2 and H358 Cell line with an IC50 of ~>30 uM,[5] which we assume to be a reason for lack of synergy

Conclusion

- **IND126** is a potent, covalent KRASG12C inhibitor that selectively inhibited KRAS-dependent signal transduction and viability in KRASG12C mutant cell lines.
- **~4- fold** increase in pEGFR inhibition was observed upon addition of 30 nM of IND126 to Afatinib, indicating KRASG12C inhibitor may increase the sensitivity of EGFR inhibitors in KRASG12C mutant cells lines.
- **>50 %** Inhibition was observed with combination of 278 nM of Palbociclib and 41 nM of IND126 with a Composite Synergy Score of 5+, indicating significant synergy.
- **IND126** synergistically potentiates activity of both Afatinib and Palbociclib in H358 and MIAPaCa-2 cell lines with high degree of synergy, while there was no synergy observed with Alpelisib.
- Our data demonstrate use of KRASG12C inhibitor, **IND126** in combination with EGFR and CDK4/6 inhibitors may lead to better outcomes and a possible means to overcome any acquired resistance with KRASG12C and/or EGRF inhibitors as single agent.
- Combination of **IND126**, with inhibitors of Ras/Raf/MEK/TKI pathways could be an effective approach to overcome the acquired resistance and/or reactivation of alternative pathways.

References

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3) Neumann et al., Pathol Res Pract 2009;205(12).
4) Arbour KC, et.al. Clin Cancer Res. 2021 Apr 15; 27(8): 2209–2215.
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