When Light Matters: Exploring Light Exposure and Stability Risk in Early-Stage Development of ADCs

Amaryllis Kanyabwero*, W. Aaron Pruett*, Madeline Kolb*, Neha Sahni#, Arun Alphonse Ignatius*

Introduction

- Antibody drug conjugates (ADCs) consist of a monoclonal antibody (mAb) and a cytotoxic payload attached via a linker.
- Some ADCs require extensive assessment of product stability due to unique degradation pathways that can be introduced by light exposure.
- Payload drugs such as camptothecins (refer to Figure 2) can be photoreactive which can lead to photo-induced chemical modifications in the ADC including oxidation and aggregation.
- Visible light and UV light can oxidize the payload leading to the formation of reactive oxygen species or self-photolysis.

Figure 1: Pathways for photodegradation of photoreactive ADC (Adapted from Ref. 1)

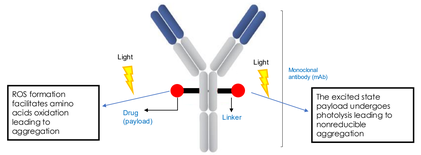
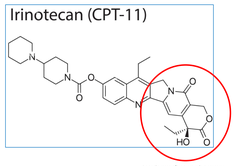
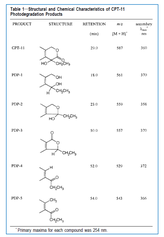


Figure 2: Structure of photoreactive payload and its photo degradants (Adapted from Ref. 2)





Methods

- Photostability studies are typically performed during late-stage pharmaceutical development and follow ICH guidelines which recommend illumination of 1.2 million lux hours and 200 W/m^2 of near UV light exposure.
- However, some drugs such as ADCs may require evaluation and environmental protections in early preclinical development.
- A limited scope study mimicking worst case indoor light during product development and manufacturing was performed to assess the impact of light exposure on the stability of three ADCs and address relevant light exposure risks.

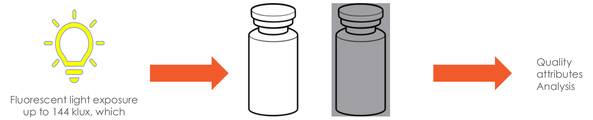


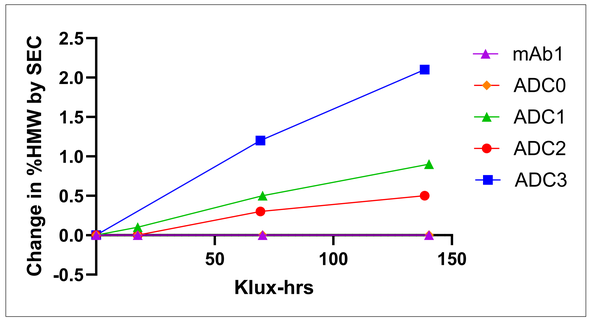
Table 1: Methods used for quality attribute testing

Attribute	Test Method
Visual Inspection	Appearance
Charge heterogeneity	IE-HPLC
Aggregation	SE-UPLC
Thermostability	DSC
Intact Mass and primary structure	LC-MS Peptide mapping

Physiochemical Changes (SEC, IEC)

- Photooxidation under light exposure can primarily lead to aggregation and changes in charge profile.
- Differences in HMW species formation were observed upon light exposure across various ADCs.
- No changes in HMW species were observed in the dark control samples.

Figure 3: Comparison of mAb and ADCs in clear vials: change in %HMWS



- Changes in charge variants were observed for all 3 ADCs when exposed to light.
- A decrease in % main peak and a subsequent increase % acidic variants was observed for light exposed ADCs. No differences in charge profiles were observed in dark control samples.

Figure 4a: Comparison of mAb and ADCs in clear vials: change in % acidic species

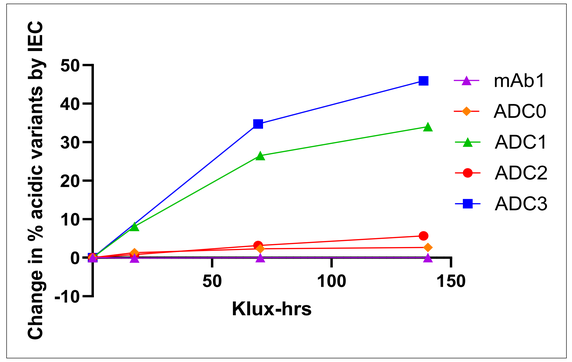
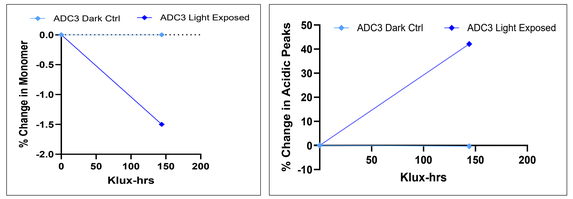


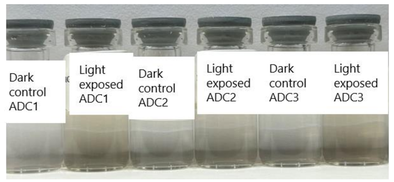
Figure 4b: Comparison of representation light exposed ADC and the dark control: change in %HMW species and change in % acidic peaks



Visual Appearance

- Visual Appearance results show a color change under light exposure stress.
- All light exposed ADCs had a color similar to the light brown (B6/B5) standard.
- Samples kept in the dark had no change in color and remained similar to the colorless (B9) standard.
- No changes to turbidity or visible particles were observed.

Figure 5: Representative images of color change for 3 ADCs after light exposure (144klux-hours)



Biophysical Characterization

- Overall structure was assessed using a differential scanning calorimeter (DSC). The samples were scanned from 10–95°C at a scan rate of 60°C/hr and the thermal transition midpoints were obtained from peak maximum values.
- Some changes in Tm1 transitions were observed for all ADCs upon light exposure compared to the dark control samples.

Figure 6: Overall conformational stability for light exposed and dark control ADC1, ADC2 and ADC3

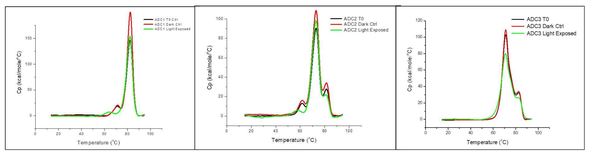
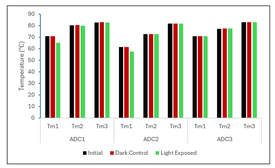


Figure 7: Thermal Melting temperatures in light exposed ADCs compared to the dark control ADCs



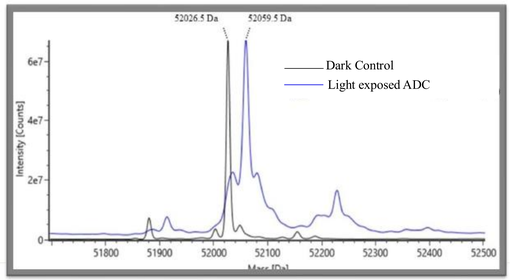
Conclusions

- A preliminary assessment of photostability during early formulation development can identify stability risks during formulation and manufacturing of ADCs.
- Photosensitivity in ADCs can vary greatly depending on the antibody/payload combination.
- Photostability risk can be managed through controls during manufacturing and administration. Mitigation strategies in early development could include:
  - Simple controls in manufacturing settings such as yellow or dim lights and process controls to reduce overall light exposure
  - Implementation of light protective primary packaging such as amber vials and use of opaque IV bag sleeves in clinical settings

Mass Spectrometry

- Intact mass spectrometry analysis was conducted to assess differences in profile for ADCs upon light exposure.
- The reduced profiles of the light stressed ADC show a shift in mass for the heavy chain of ~32 Da compared to the dark control suggesting oxidation.
- The non-reduced profiles also showed a shift in mass heterogeneity in ADC samples after light exposure (data not shown).

Figure 8: Representative chromatogram of an ADC with light exposure (blue) and without light exposure stress (black)



- Peptide mapping was conducted to assess the modifications in photo-stressed ADC samples.
- Changes in %oxidation of Met residues were observed, resulting in ~60-70% increase in oxidation.

Table 2: Reduced peptide mapping on an ADC showing %oxidation for the light stress ADC and the dark control ADC

Peptide	Site/Modification	ADC3 Dark Control	ADC3 light exposed
LC	M1 %oxidation	0.9	3.8
HC	M2, W1 %oxidation	2.4	2.8
HC	M3 %oxidation	2.6	70.0
HC	M4 %oxidation	1.6	58.5
HC	W2 %oxidation	0.0	1.0

References

1. Zhou, T., Dong, X., Yu, J., Song, C., Liu, Q., He, L., Chen, W., Luo, W., Song, J., Su, Y., Pan, J., & Xu, A. (2025). Photostability of Topoisomerase I Inhibitor Conjugated IgG1 Antibody-Drug Conjugates: Characterization Study and Degradation Mechanism Analysis. Bioconjugate chemistry, 36(12), 2645–2654. https://doi.org/10.1021/acs.bioconjchem.5c00485
2. Dodds, H. M., Craik, D. J., & Rivory, L. P. (1997). Photodegradation of irinotecan (CPT-11) in aqueous solutions: identification of fluorescent products and influence of solution composition. Journal of pharmaceutical sciences, 86(12), 1410–1416. https://doi.org/10.1021/js970110c

Acknowledgements

- This work was completed prior to the MacroGenics and Bora Pharmaceuticals transition.
- *Currently at Bora Pharmaceuticals; #Currently at MacroGenics