Quantitation of released and total AZD4320 from Nanomedicine Dendrimer by LC-MS/MS

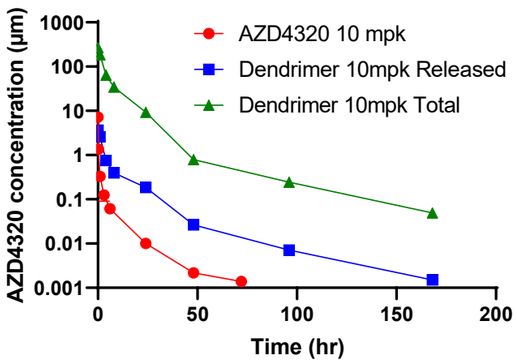
Zhang, Sunny (Guangnong); Gibbons, Francis D.; Beaudoin, Marie-Eve; Pop-Damkov, Petar; Wild, Martin; Balachander, Srividya; Fretland, Adrian J.; Gangl, Eric

Oncology R&D, Research and Early Development, AstraZeneca Pharmaceuticals, Waltham, MA, USA 02451

Introduction

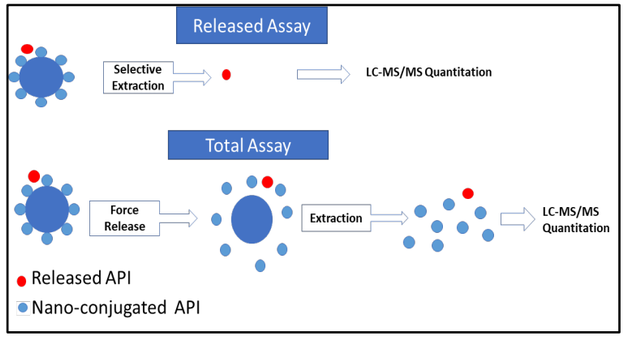
AZD4320 chemical structure (left) and Dendrimer (right)

- AZD4320 is a potent dual Bcl-2/xL inhibitor developed for the treatment of hematologic and solid tumors.
- An innovative drug delivery PEGylated dendrimer was developed to improve solubility and therapeutic margin.

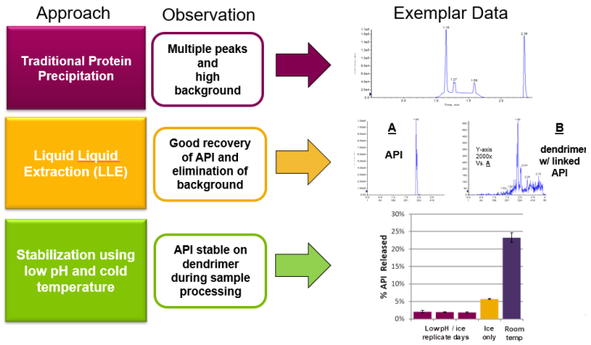


- The dendrimer nanomedicine alters the PK profile by reducing C_{max} and extending terminal half life.
- Reliable measurement of the released and "total" drug concentration is critical to understand the drug exposure and to assist in PK/PD model development.

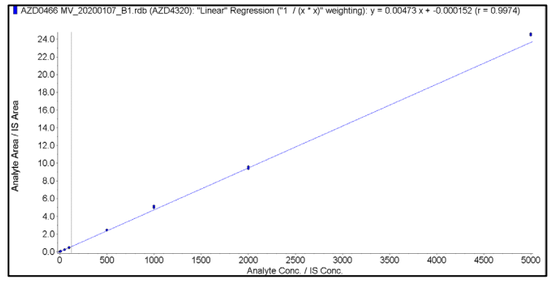
Bioanalysis Challenges/Solutions



- **FDA Nanomedicine (NM) Bioanalysis Draft Guidance** states "In general, *total*, *free*, and nanomaterial-associated drug should be measured separately or indirectly derived.
- Total = released (*free*) + nanomaterial-associated
- **Released assay challenge:** selectively extract released drug without post-collection release.
- **Total assay challenge:** completely cleaved the nanomaterial –associated drug from the dendrimer.



Results



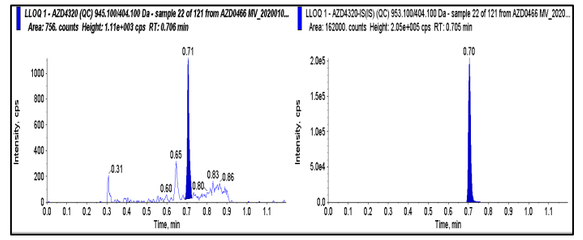
Calibration Curve and linearity (R^2>0.98)

- **Released assay:** 1-5000 ng/mL (API standard curve)
- **Total assay:** 10-25,000 ng/mL (NM standard curve)

	Assayed Released AZD4320 concentration (ng/mL)				
	API AZD4320 Spiked			Dendrimer Spiked	
	LLOQ	LQC	HQC2	Nano_LQC	Nano-HQC
	1.00	3.00	4500	1500 (total)	150,000 (total)
Rep1	1.02	2.35	4530	6.81	1020
Rep2	0.857	2.41	4590	6.48	999
Rep3	0.871	2.64	4330	6.64	1020
Rep4	0.878	2.57	4370	8.61	1020
Rep5	0.865	2.34	4540	6.44	1010
Rep6	0.998	2.35	4640	6.45	1000
n	6	6	6	6	6
mean	0.915	2.44	4500	6.91	1012
SD	0.0736	0.130	123	0.847	10.1
% CV	8.0	5.3	2.7	12.3	1.0
% Bias	-8.5	-18.6	0.0	NA	NA

Accuracy and Precision

- API QCs demonstrate good accuracy and precision with % bias and %CV < 20%.
- NM QCs shows good precision with %CV < 20%. Absolute accuracy of NM QC isn't achievable due to lack of an authentic standard for NM starting material.



- **Sensitivity**: LLOQ at 1 ng/mL.
- **Selectivity**: Unique PPT-LLE extraction method resulting in clean background.

	Assayed Released AZD4320 concentration (ng/mL)					
	Dendrimer spiked in plasma					
	Nano_LQC (1500 ng/mL total)			Nano_HQC (150,000 ng/mL total)		
	T0	Ice 2hr	F/T	T=0	Ice 2hr	F/T
Rep1	7.70	7.63	10.2	1270	1210	1760
Rep2	8.26	8.24	9.75	1240	1310	1670
Rep3	8.04	7.31	10.5	1190	1370	1590
n	3	3	3	3	3	3
Mean	8.00	7.73	10.2	1233	1297	1673
SD	0.28	0.47	0.38	40.4	80.8	85.0
CV %	3.5	6.1	3.7	3.3	6.2	5.1
Diff vs T0 (%)		-1.7	11.8		2.5	15.1
F/T: Freeze/thaw -20ºC, 2 cycles						

Released drug stability assessment

- Citric acid was added during sample collection to control post-collection release.
- Low temperature and pH are applied during the bench-processing step.
- Stability was assessed by comparison with baseline release of fresh prepared dendrimer QCs from T0.

Bioanalysis Method details

The released and total drug concentration in biological matrices were determined using a protein precipitation/liquid-liquid extraction (PPT-LLE) procedure followed by liquid chromatography tandem mass spectrometric detection (LC-MS/MS). Subsequent extraction procedures were the same for both released and total assays.

Calibration Curve and QCs Preparation

- AZD4320 and dendrimer calibration standards were prepared in mouse plasma (1-5,000 ng/mL for released and 10-25,000 ng/mL for total assay).
- AZD4320 and dendrimer quality control samples were prepared in plasma.

Total assay with hydrazine incubation

- Samples are flash-frozen upon collection without stabilizer. Samples are subsequently incubated with hydrazine to disrupt the nanoparticles, causing conjugated drug to be released.
- 25 µL of unknown sample is added to 100 µL of 2 M of hydrazine solution and then incubate at 37°C for ~16 hours.
- Following incubation, transfer 25 uL of released sample to a 96-well plate and mix with 25 uL of plasma with 1M citric acid, and then follow the released assay extraction.

Released assay extraction (PPT-LLE)

- Samples are stabilized by addition of citric acid and chilling on ice upon collection. This approach stops post-collection release.
- Add 225 uL of IS in water/MeOH/ACN (75/15/135 v/v/v) to 50 µL of samples, with 5% formic acid to the plate. Gently mix.
- Add 300 µL of 10:90 cyclohexane : ethyl acetate, Cover the plate with a plastic mat, shake vigorously (~5min) and then centrifuge at 4000 rpm for 5 min.
- Transfer 300 µL of upper layer and dry down completely under nitrogen.
- Reconstitute with 200 uL of 5% formic acid in acetonitrile and submit for LC-MS/MS analysis.

LC-MS/MS

UPLC: Shimadzu LC-30AD
Mass Spectrometer: AB Sciex Triple Quad 6500
Column: Waters Xbridge C18, 2.1 x 30 mm, 3.5 µm
Mobile Phase A: 10 mM Ammonium Formate with 0.1% formic acid
Mobile phase B: 0.1% formic acid in Acetonitrile
Gradient: 35-95% B over 1.2 min

Analyte	Q1 m/z	Q3 m/z	Time (ms)	CE
AZD4320	945.1	404.1	75	60
^{13}C-AZD4320-d_7 (IS)	953.1	404.1	25	60

Novel Aspects/Conclusion

- Each nanomedicine has unique bioanalytical challenges. A general released and total assay strategy was created.
- Specific and highly sensitive methods were developed to determine the released and total concentration of AZD4320 from its dendrimer formulation in biological matrices. These methods are the first novel practical approaches to measure released and total separately from nanomaterial dendrimer.
- The overall concepts such as using the nanomaterials QCs to monitor the control of release in released assay and to track the forced release completion in the total assay, as well as using stabilizer to prevent/control nanomedicine post-collection release may serve as a paradigm for future nanomaterial analysis.

Acknowledgements
BCL2 Project Team, Yan Li, Shenghua Wen, Areya Tabatabai

Supported by AstraZeneca IMED Biotech Unit

Presented at The 2020 14th Workshop on Recent Issues in Bioanalysis (WRIB) , 15-29 June 2020