Imaging Flow Cytometric Detection of amp(1q21) and del(17p) "Double-Hit" Abnormalities in Myeloma Plasma Cells

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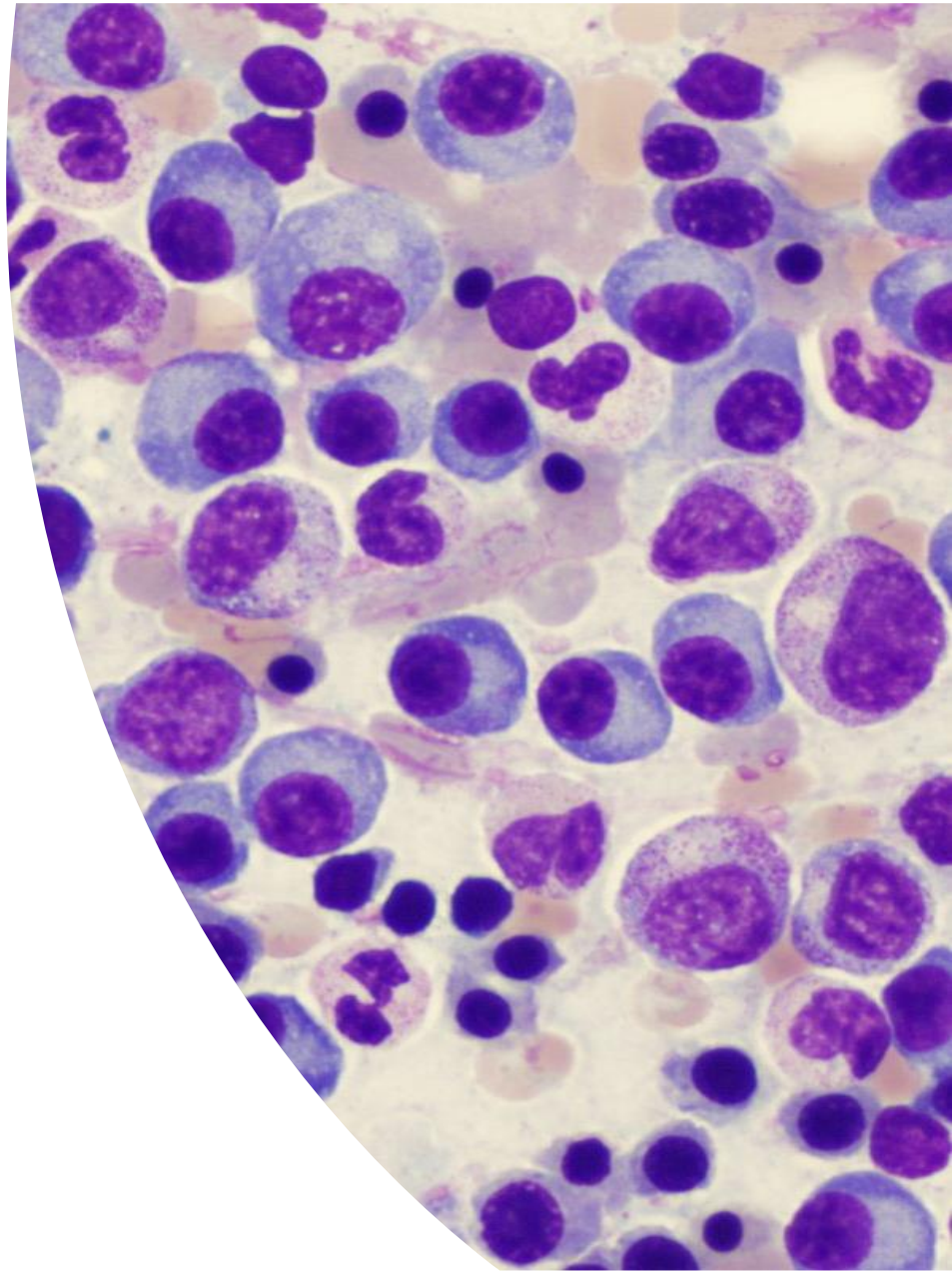
Multiple Myeloma

- Plasma cell neoplasm in the bone marrow
- Disseminated with circulating disease
- 3rd most common haematological malignancy (175,000 new cases pa globally)
- Most common in people >65 years
- Treatable but incurable
- 5-year overall survival rate ~50%



Cytogenetic Abnormalities

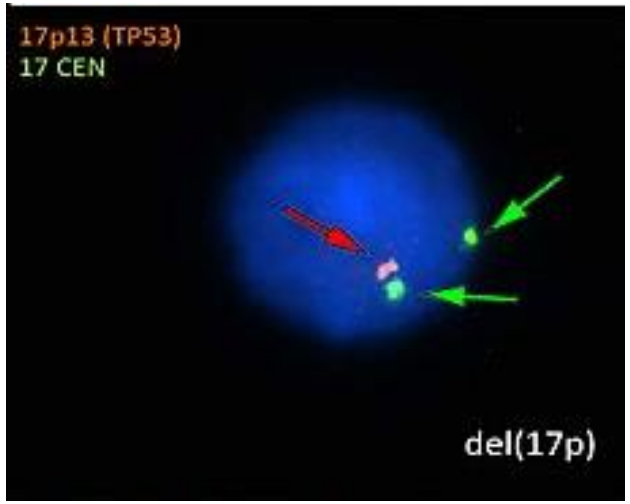
Detected by FISH on bone marrow: 200 nuclei assessed

Primary abnormalities

- Trisomies (odd numbered chromosomes)
- Translocations of the *IgH* locus (14q32)

Secondary abnormalities

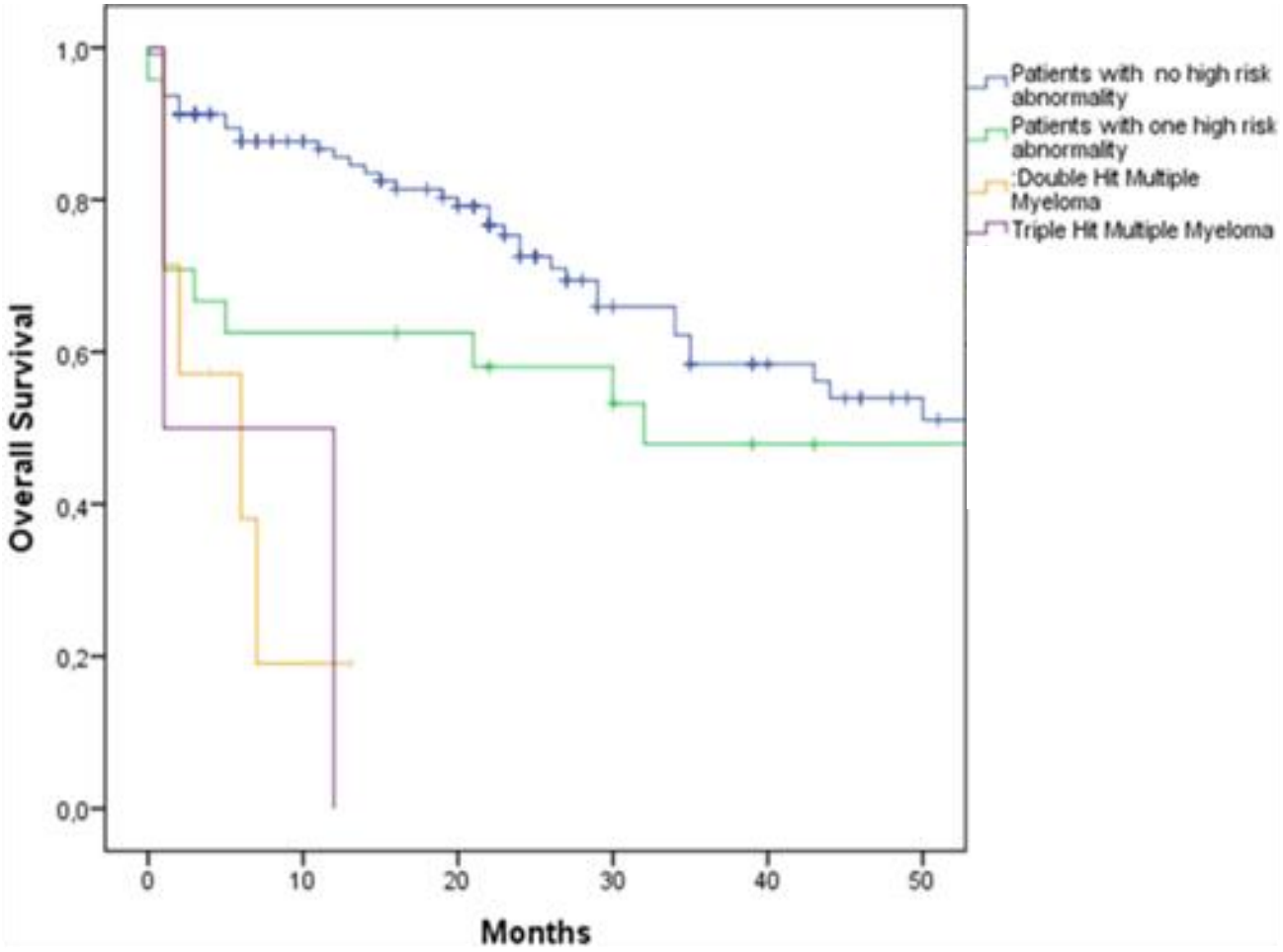
- Amplification and deletions



Risk Category	Cytogenetic Abnormalities
Standard-Risk	Trisomy 3, 5, 7, 9, 11, 15 ,19, 21 t(6;14); t(11;14)
High-Risk	t(4;14); t(14;16); t(14;20) del(17p); amp(1q21)

‘Multiple Hits’

- ‘Double hit’
  - Presence of two high-risk abnormalities
  - amp(1q21) and del(17p) most significant prognostically
- ‘Triple Hit’
  - Presence of three high-risk abnormalities



Baysal, M et al. Sci Rep. 2020.

Aims and Methods

- To determine whether amp(1q21) and del(17p) colocalise or are in discrete cells
- To assess using a novel imaging flow cytometric approach.

Methods

- Blood and bone marrow from myeloma patients
- Analysis by “immuno-flowFISH”

“Immuno-flowFISH”

- UWA invention to detect chromosomes in cells identified by the immunophenotype
- Developed for assessment of CLL
  (Hui, H et al. Cytometry A. 2019)
- Imaging flow cytometer
  (Cytek AMNIS ImageStreamX mkII)
- High throughput automated analysis
  (500-2,000 cells/sec)

60x Magnification

Extended Depth of Field (EDF)

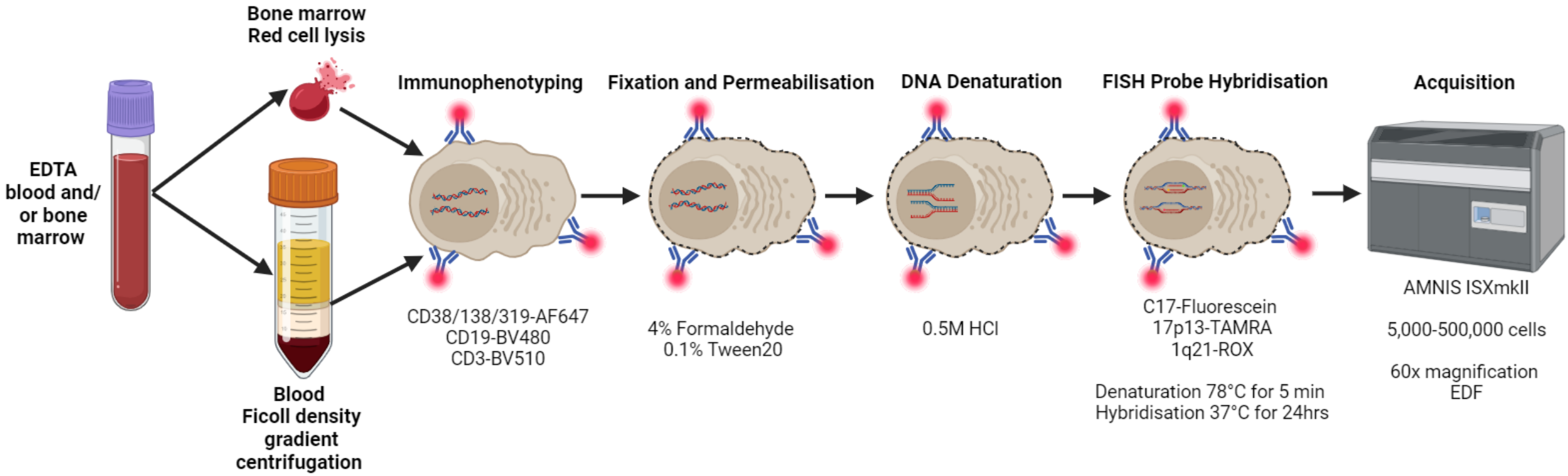
2x CCND Cameras

6 lasers:

405nm, 488nm, 561nm,
592nm, 642nm, 785nm

The Immuno-flowFISH Method

- Assessed 32 patients
  - 24 bone marrow
  - 19 blood



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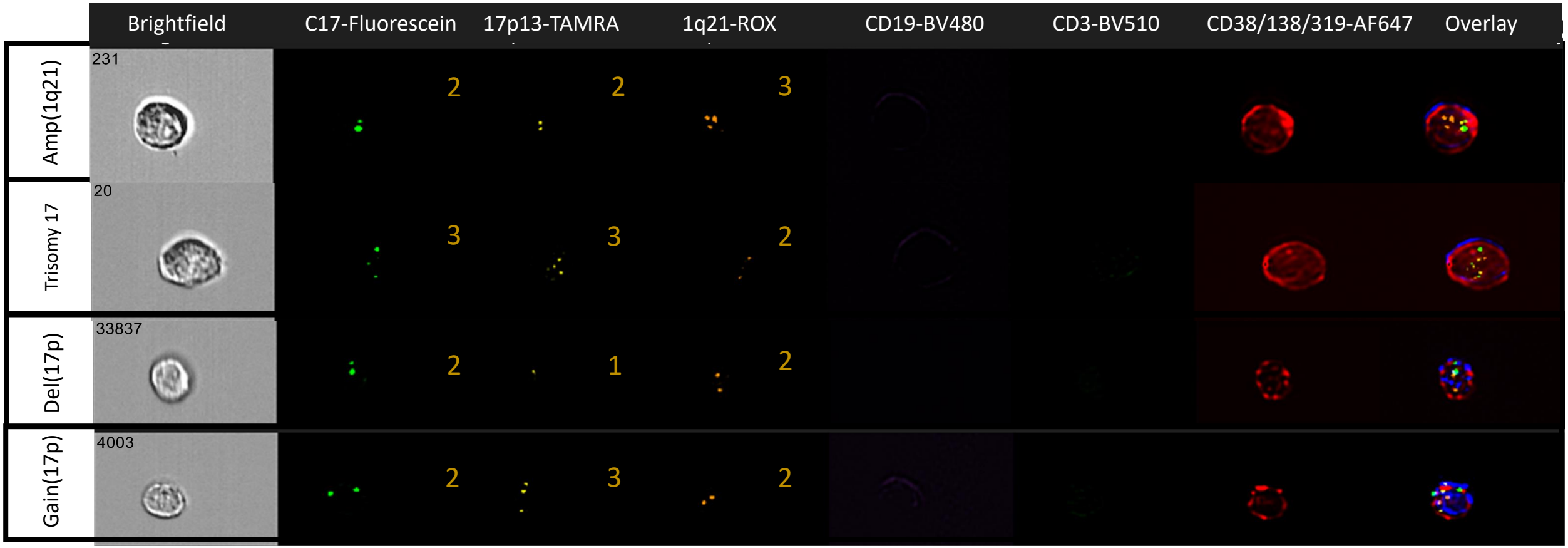
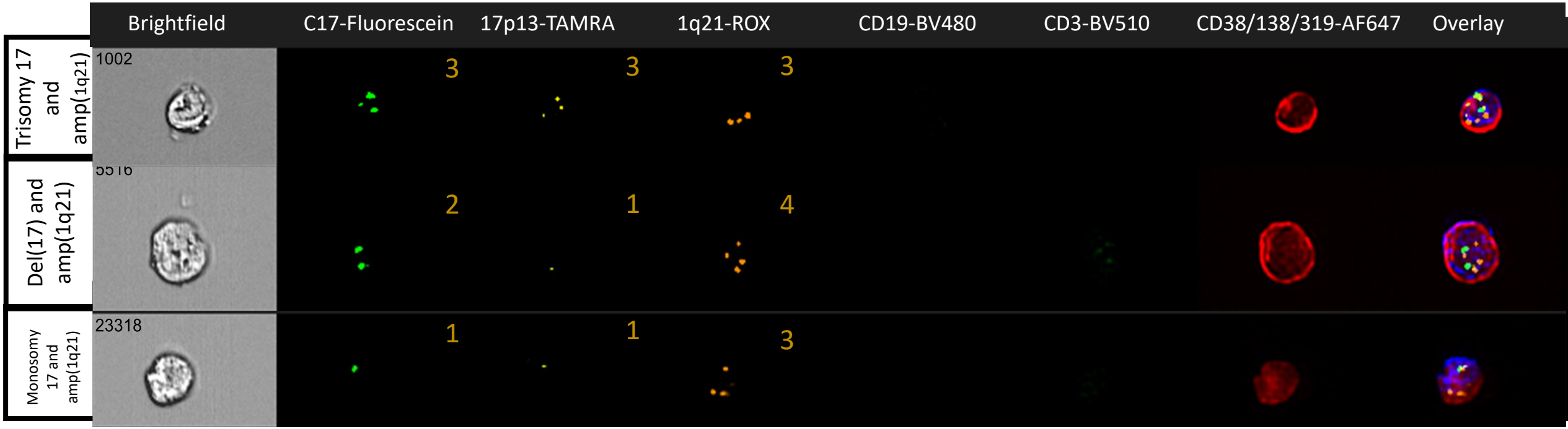
Individual Abnormalities



- 6 patients with amp(1q21)
- 5 patients Chr17 abnormalities

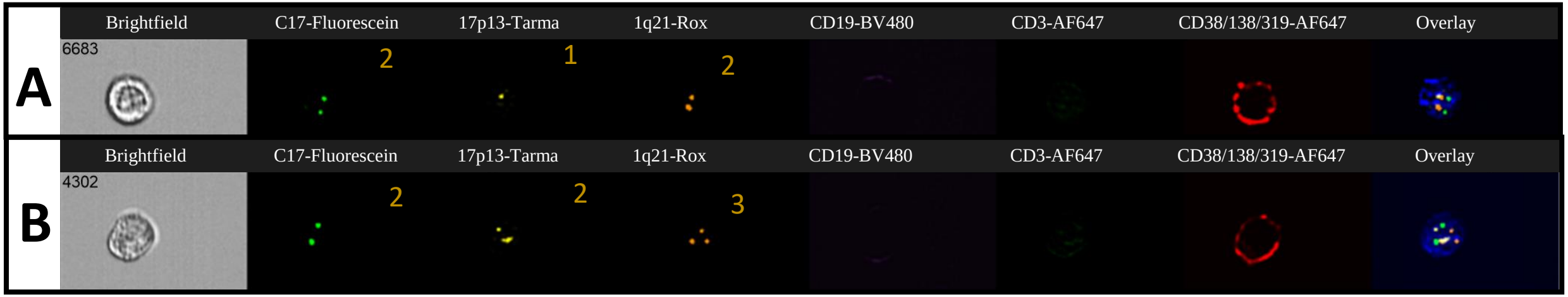
Double Hit Abnormalities



- 8 patients with Chr1 and Chr17 abnormalities

‘Double Hit’ Presentations

Patient 1



Patient 2

Patient 1

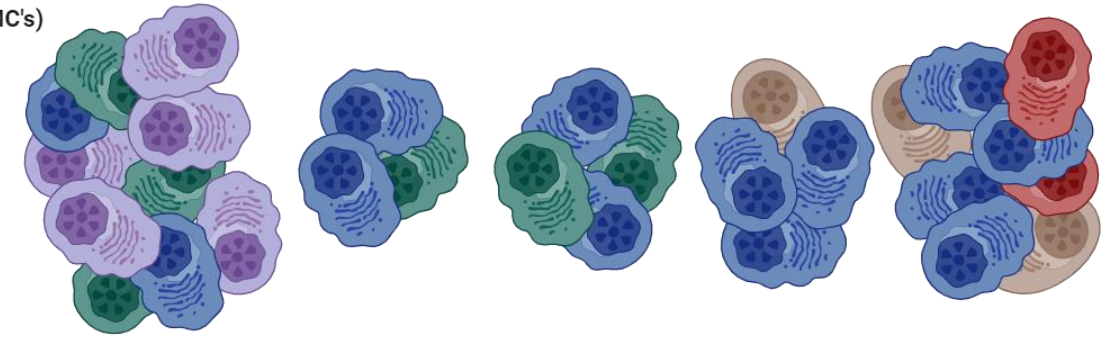
Treatments

Daratumumab, Bortezomib, Dexamethasone (DVd) Treatment

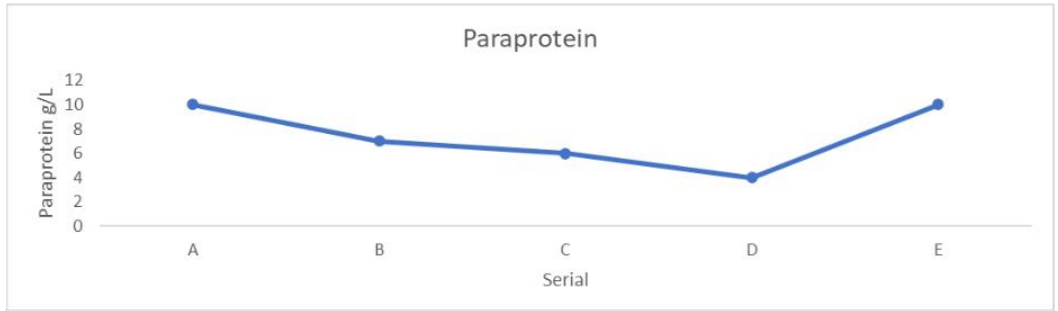
Daratumumab maintenance Treatment

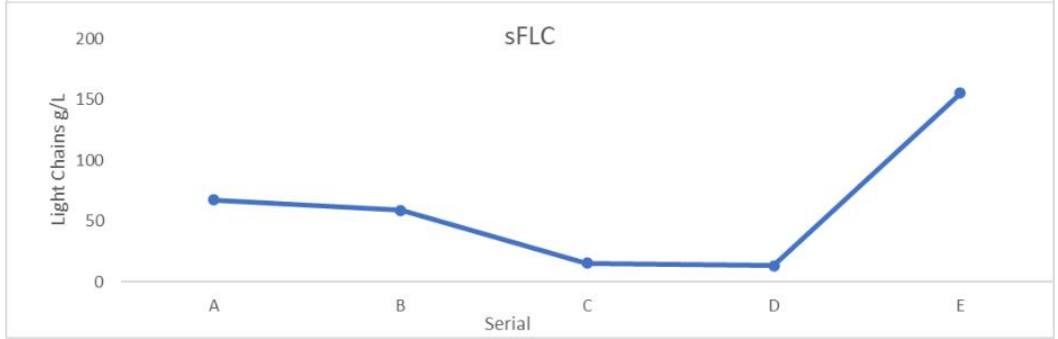
Immuno-flowFISH Monitoring

Sample:	A	B	C	D	E
% Myeloma Plasma cells of Blood PBMC's	0.074	0.003	0.004	0.004	0.048
(CD19+ Plasma Cells of Blood PBMC's)	(0)	(0)	(0)	(0.002)	(0.001)



Clinical Monitoring





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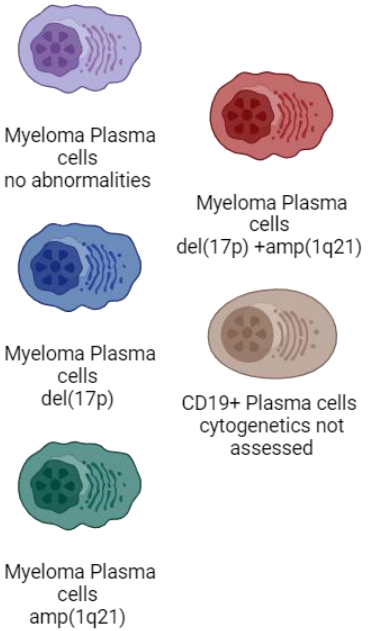

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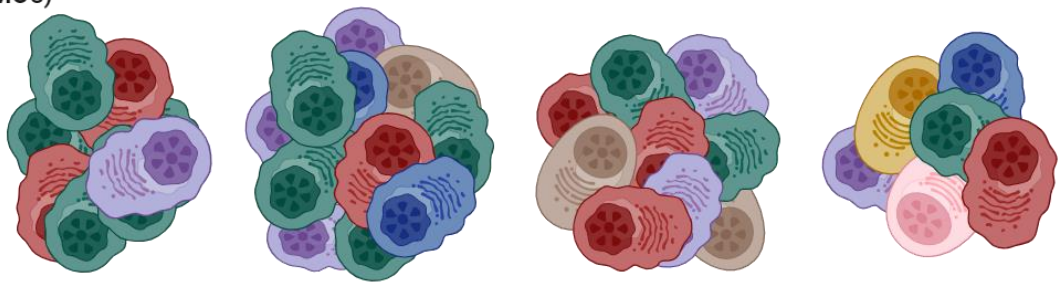
Patient 2

Treatments

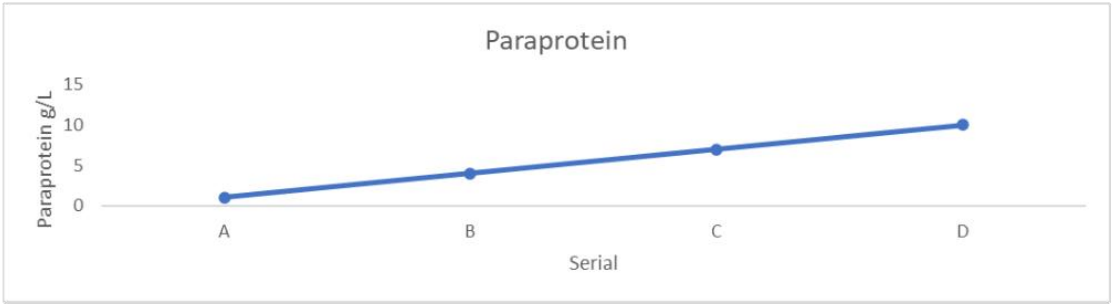
Daratumumab Maintenance treatment (Post DVd)

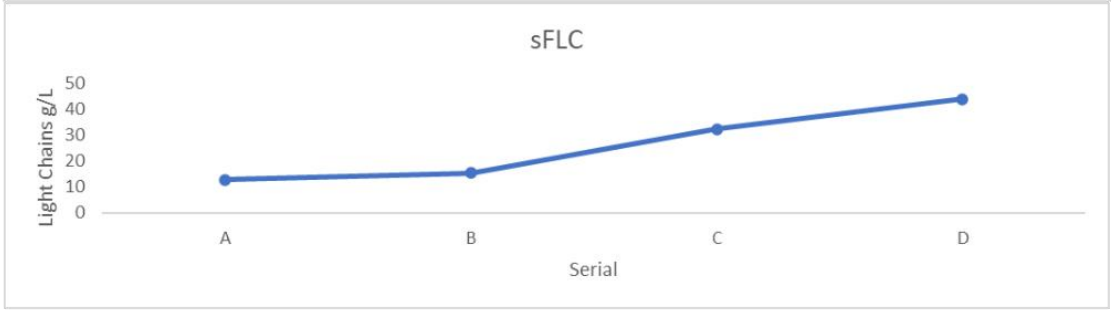
Immuno-flowFISH Monitoring

Sample:	A	B	C	D
% Myeloma Plasma cells of Blood PBMC's	0.014	0.050	0.025	0.010
(CD19+ plasma cells of Blood PBMC's)	(0)	(0.0031)	(0.0042)	(0.0048)



Clinical Monitoring





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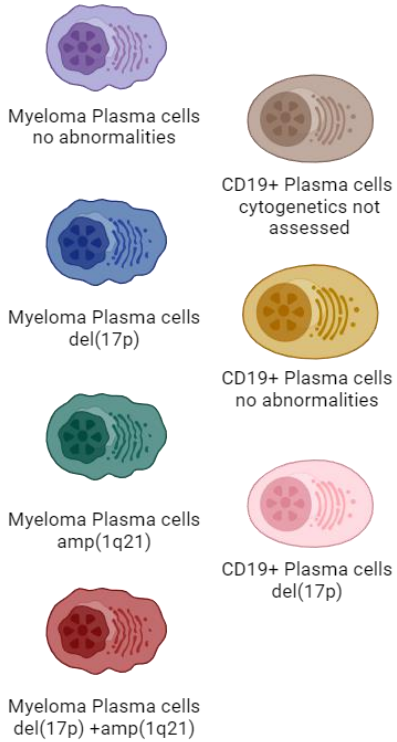

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Summary and Conclusions

- amp(1q21) and del(17p) abnormalities can be detected simultaneously by immuno-flowFISH
- Colocalised amp(1q21) and del(17p) in individual plasma cells in 6 cases indicating true “double hit” myeloma.
- Sequential time-course monitoring showed alterations in the clonal makeup with both eradication of clones and emergence of new “double hit” clones at relapse.
- Lowest limit of Detection of $1x10^{-5}$
- Blood monitoring abnormalities may facilitate patient care

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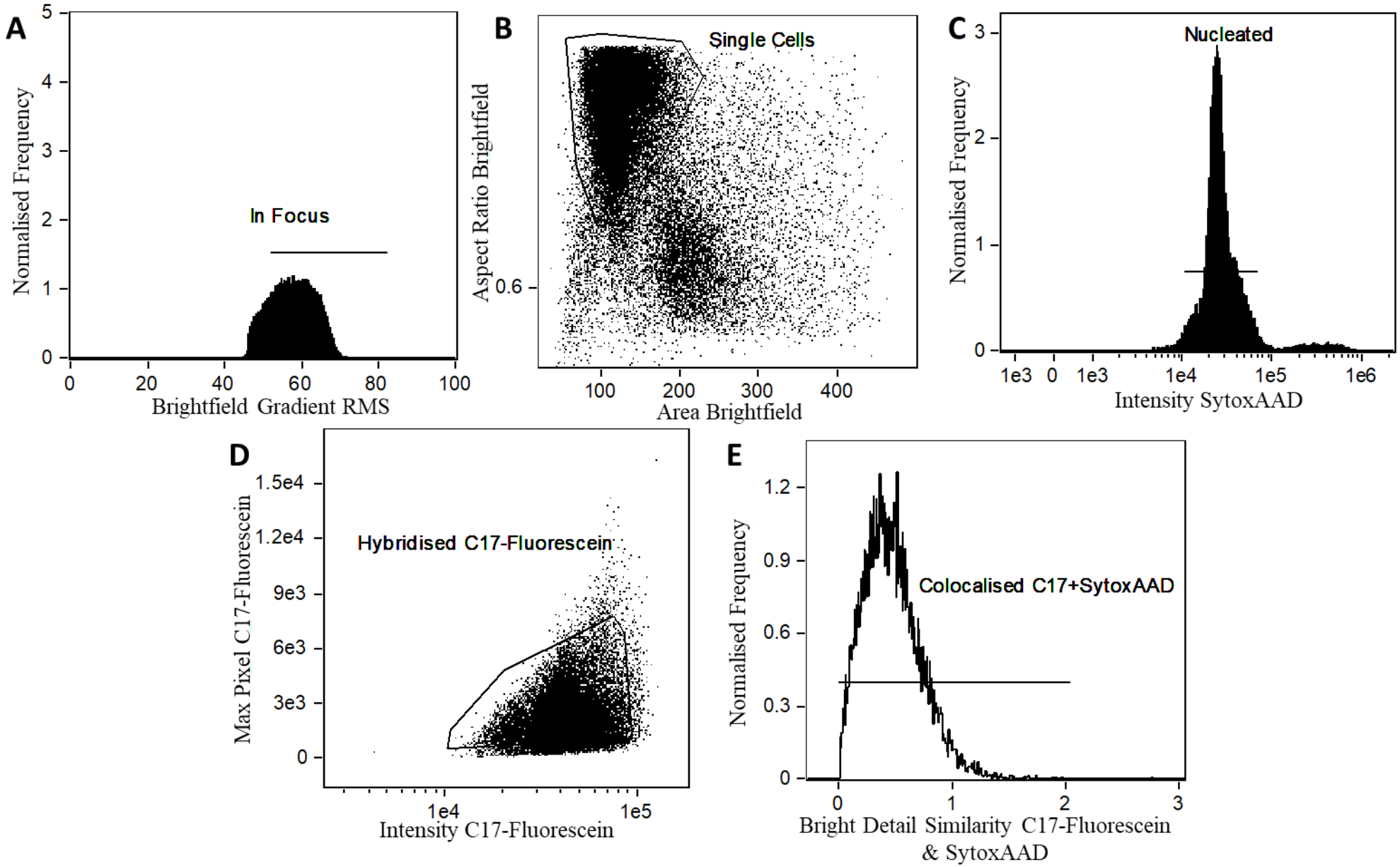
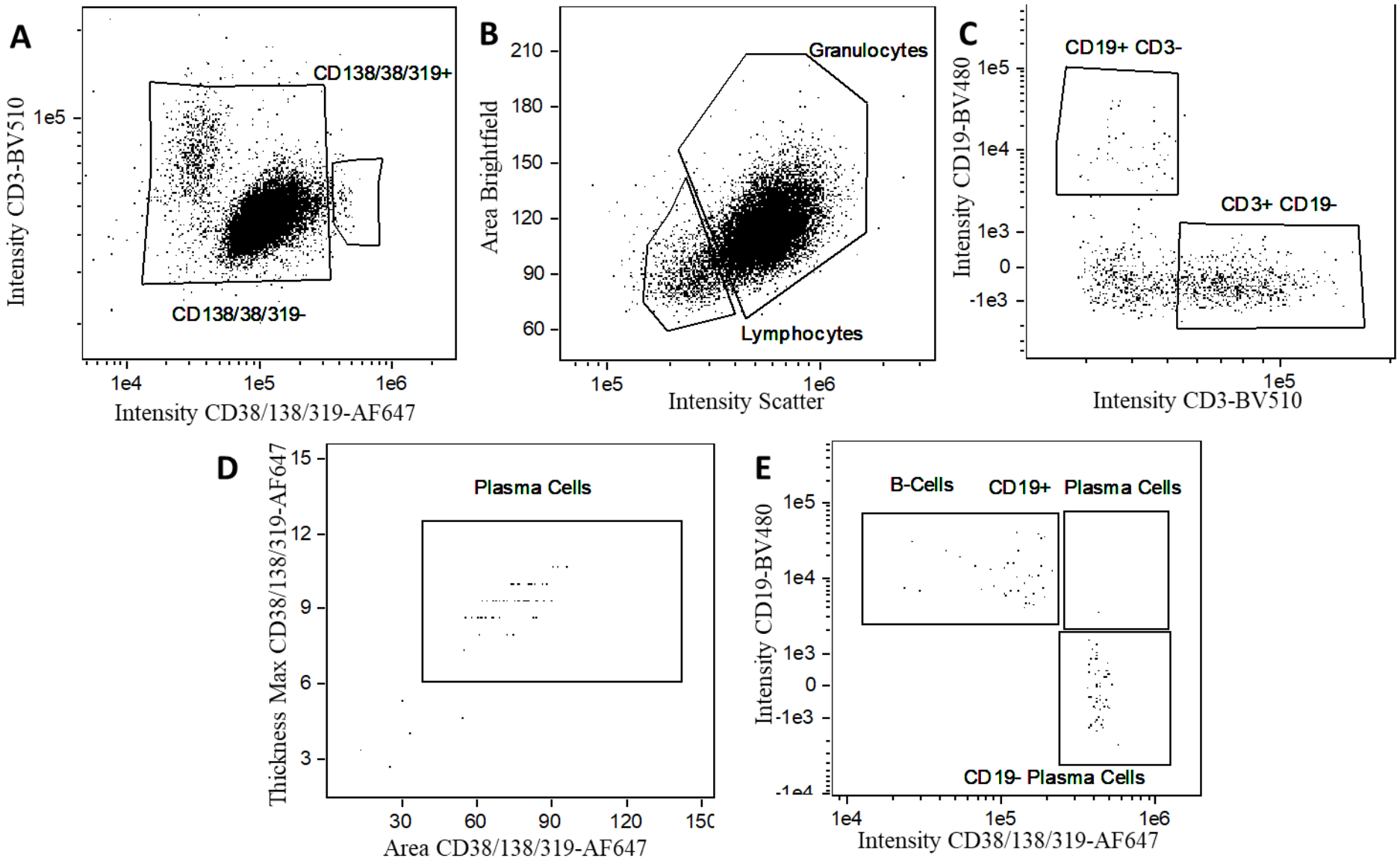

A
In Focus
Normalised Frequency
Brightfield Gradient RMS
B
Single Cells
Aspect Ratio Brightfield
Area Brightfield
C
Nucleated
Normalised Frequency
Intensity SytoxAAD
D
Hybridised C17-Fluorescein
Max Pixel C17-Fluorescein
Intensity C17-Fluorescein
E
Colocalised C17+SytoxAAD
Normalised Frequency
Bright Detail Similarity C17-Fluorescein & SytoxAAD


A
CD138/38/319+
CD138/38/319-
Intensity CD3-BV510
1e5
1e4
1e5
1e6
Intensity CD38/138/319-AF647
B
Granulocytes
Lymphocytes
Area Brightfield
210
180
150
120
90
60
1e5
1e6
Intensity Scatter
C
CD19+ CD3-
CD3+ CD19-
Intensity CD19-BV480
1e5
1e4
1e3
0
-1e3
1e5
Intensity CD3-BV510
D
Plasma Cells
Thickness Max CD38/138/319-AF647
15
12
9
6
3
30
60
90
120
150
Area CD38/138/319-AF647
E
B-Cells
CD19+ Plasma Cells
CD19- Plasma Cells
Intensity CD19-BV480
1e5
1e4
1e3
0
-1e3
1e4
1e5
1e6
Intensity CD38/138/319-AF647

Establishing Confidence Intervals

Confidence Intervals			
Probe	C17	17p13	1q21
Mean	1.027	1.016	1.024
Standard Deviation	0.019	0.028	0.022
Lower 95% CI	0.990	0.961	0.982
Upper 95% CI	1.063	1.071	1.067