Současné možnosti vyšetřování cytogenetických změn u mnohočetného myelomu

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Přírodovědecká fakulta MU

Univerzitní výzkumné centrum – Česká myelomová skupina

FN BRNO

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Nejčastější cytogenetické nálezy u pacientů s MM

Početní změny chromozomů

Hypodiploidie: nejčastěji monozomie chromozomu 8, 13, 14, 22, X
nepříznivá prognóza

Hyperdiploidie: nejčastěji trizomie chromozomu 3, 5, 7, 9, 11,
příznivá prognóza

Strukturní změny chromozomů

Translokace zahrnující IgH gen (14q32):
t(11;14) (q13;q32)
t(4;14)(p16;q32), t(14;16) (q32;q23) **nepříznivá prognóza**

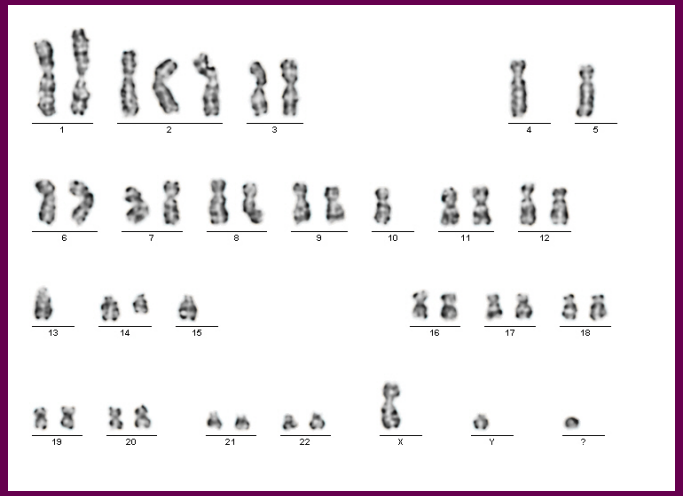
Delece genu *RB1* (13q14) ***střední prognóza***

Delece genu *p53* (17p) ***nepříznivá prognóza***

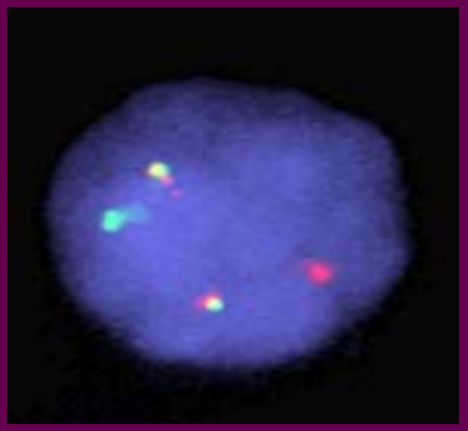
Zisk 1q ***nepříznivá prognóza***

Cytogenetické metody využitelné u MM

Metafázní analýzy

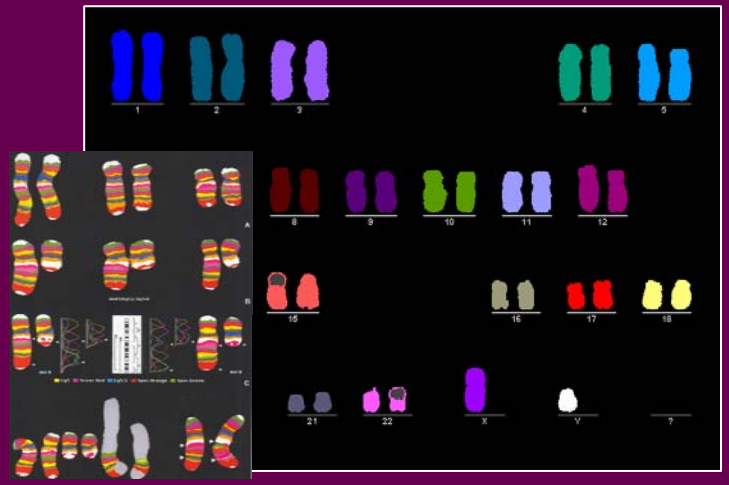


Interfázní analýzy

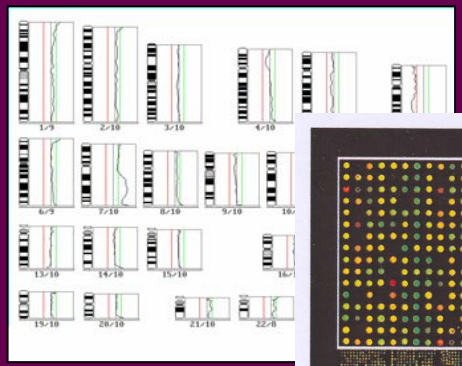


Analýzy DNA

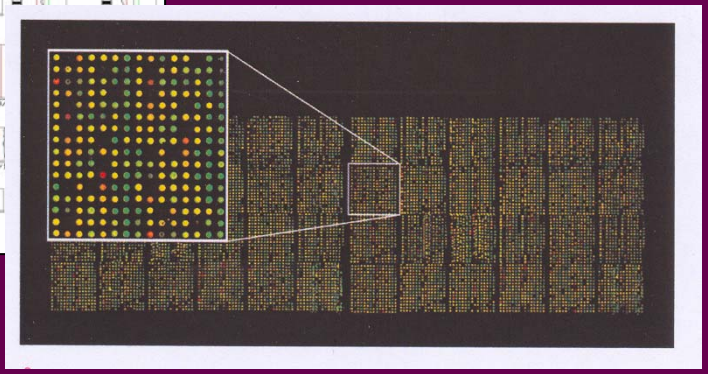
mFISH



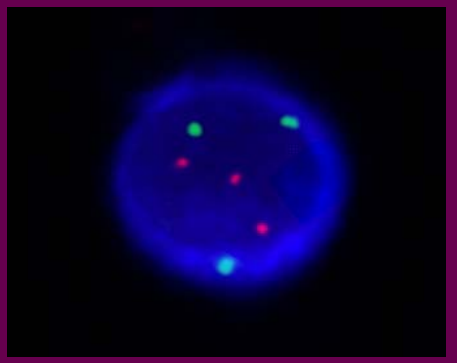
CGH



Array-CGH



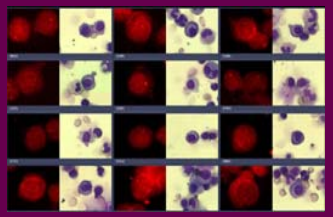
Interfázní cytogenetika u MM



Problémy

Nutná **separace** či **identifikace** myelomových buněk v KD !!!

Řešení



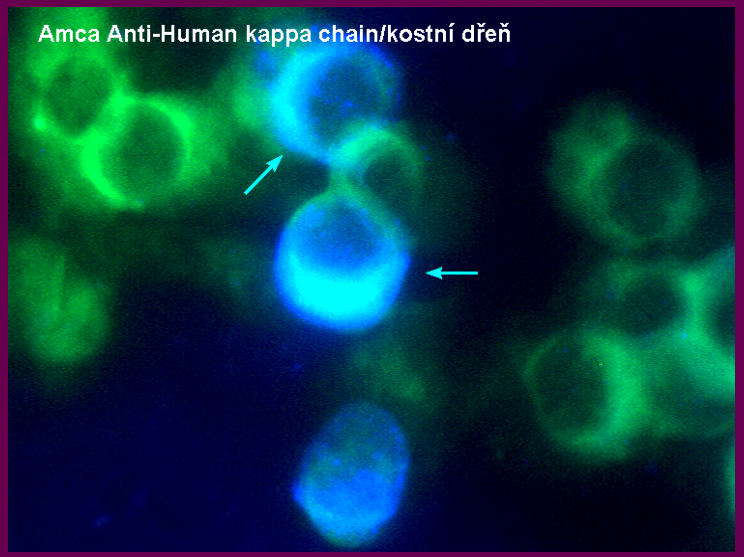
a) separace nádorových buněk z KD pomocí MACS, FACS +vyšetření technikou I-FISH

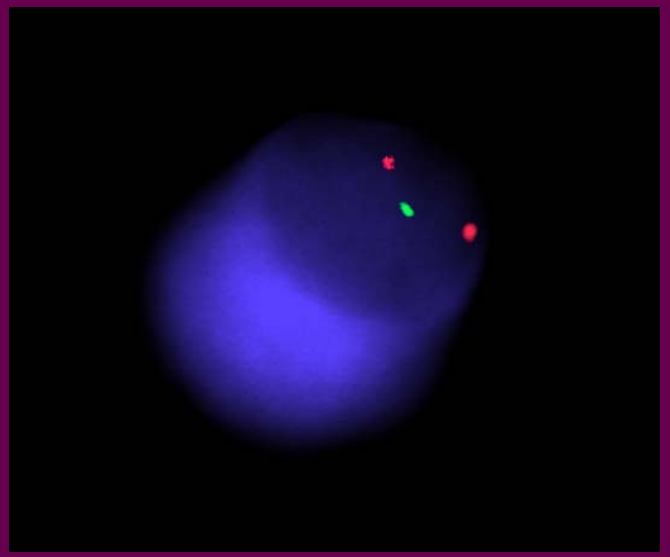
b) kombinace morfologické identifikace nádorových buněk v KD + cytogenetického vyšetření technikou I-FISH (Duet systém)

c) imunofluorescenční značení nádorových buněk v KD + vyšetření technikou I-FISH (cIg FISH)

Imunofluorescenční detekce myelomových buněk a interfázní cytogenetika

Zavedení a standardizace techniky cIg – FISH v ČR **2003**





- **Ahmann GJ, Jalal SM, Juneau AL, et al.** (1998): A novel three-color, clone-specific fluorescence in situ hybridization procedure for monoclonal gammopathies. Cancer Genet Cytogenet. 101: 7-11
- **J. Drach:** personal communication

Čejkovice, 2003

Molekulárně cytogenetická analýza značených plazmatických buněk a prognostický význam klonálních chromozomových aberací u MM

Grant IGA MZ CR NR 8183-4 (2004-2007)

- Praha
- Brno
- Olomouc
- Plzeň
- Hr. Králové
- České Budějovice

- **sjednocení metodiky V ČR (cIg-FISH)**
- **vyšetření: del(13)(q14) / monozomie 13**
  t(IgH) ⇨ t(11;14)(q13;q32)
  del p53, amp 1q21
- **korelace s klinickými parametry**

Čejkovice, 2004

Standardizace cytogenetického vyšetření u MM v Evropě

EMN FISH Workshop 2005
(London, March, Royal Marsden Hospital)

24 active labs

18 also do cytogenetics
10 use purified PC
6 FISH nuclei exposed to hypotonic & Carnoy's fixative
- **2 use cytospins**
- **2 use both techniques**

20 use whole BM or mononuclear cells (6 also purified PC as above)
- **4 currently using immunostaining + FISH**
- **4 about to start this**
- **3 using morphology (but 2 apparently on cells exposed to hypotonic & Carnoy's fixative)**
- **only 5 currently using smears or cytospins!**

Standardizace cytogenetického vyšetření u MM v Evropě

EMN FISH Workshop 2005

XI International Myeloma Workshop, Kos 2007
(Ross et al., European myeloma network recommendation for FISH in myeloma)

Přijatá doporučení:

a) Materiál pro FISH – první odběr KD (purifikace plazmocytů vs. cIg-FISH)
b) Hodnoty cut–off (10% translokace, 20 % delece)
c) Užití kontrolních DNA sond
d) Počty hodnocených buněk (minimum 100)
e) Typy používaných DNA sond (Abbott, Kreatech)
f) Interpretace cytogenetických výsledků (ISCN)

Čejkovice, 2005

European Myeloma Network

RECOMMENDATIONS FOR FISH IN MYELOMA

FM Ross (1), H Avet-Loiseau (2), J Drach (3), JM Hernandez Rivas (4), and P Liebisch (5) on behalf of the European Myeloma Network FISH Working Party

1 University of Southampton, UK, 2 Institut de Biologie, Nantes , France, 3 Medizinische Universität Wien, Austria, 4 Universidad de Salamanca-CSIC, Spain, 5 University Hospital of Ulm, Germany

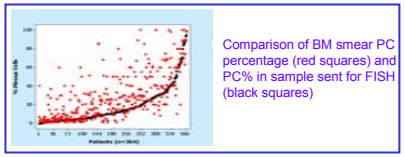
A workshop to discuss the problems particular to FISH in myeloma was held at the Royal Marsden Hospital, London on 11 March 2005 and attended by representatives of 31 European laboratories. The following recommendations are the result of agreement at the time combined with updates resulting from e-mail discussion between the participants in May 2007. The recommendations apply only to newly diagnosed or relapsed myeloma; monitoring of disease or testing of plasma cell dyscrasias with very low levels of marrow involvement may require different criteria.

Results found by applying the recommendations in this document should not yet be used to make treatment decisions except in the context of a clinical trial.

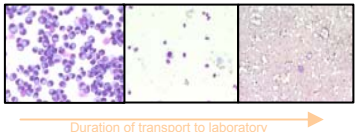
Strategies

1. It is not acceptable to report FISH results in myeloma without either concentrating the plasma cells or employing some means of plasma cell identification so that only these cells are scored. Haemodilution is a universally reported serious problem in myeloma. Clinicians should be encouraged to send part of the first draw of the aspirate for FISH, and further aspiration should involve repositioning of the needle.

2. Rapid transit to the laboratory is important. It is strongly recommended that bone marrow aspirates are not performed on Friday. Processing for FISH is time-consuming and results depend on good viability plasma cells.



Comparison of BM smear PC percentage (red squares) and PC% in sample sent for FISH (black squares)



Duration of transport to laboratory

Sample handling

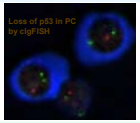
3. Purification and simultaneous immunostaining and FISH (cIgFISH) are equally valid methods. Choice of method depends on individual laboratory requirements. In general, the expense of purification is best justified in the context of a cell bank. Differences in purification methods should not affect FISH results.

4. Purified plasma cells must be checked for the proportion of plasma cells (by morphology or immunostaining)

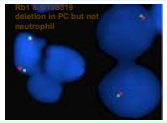
5. Purified plasma cells should be fixed in 3:1 methanol:acetic acid. The choice of doing this to the cell suspension (with or without prior hypotonic treatment) or to cytospin slides is up to the individual laboratory. Both slides and suspensions can be successfully stored at -20ºC for prolonged periods.

6. Immunostaining for light chains is recommended for cIgFISH. This gives a stronger result that CD138.

7. Cells for cIgFISH should be subjected to red cell lysis or density gradient centrifugation and fixed in 3:1 methanol:acetic acid. Slides can be made directly or the suspension stored at -20ºC. Bone marrow smears can be used for cIgFISH but only when they are very fresh.



Bystander cells can be helpful to assess hybridisation efficiency



FISH standards

8. Cut off levels should be 10% for dual fusion and break-apart probes, and 20% for numerical abnormalities and single fusion results with dual fusion probes. These are conservative figures based on mean+3SD results of 5-10 controls in several laboratories but it is recognised that suitable control material is difficult to obtain and myeloma cells are prone to artefacts, thought to be due to the paraprotein levels. Any laboratory setting up myeloma FISH should ensure that their results are compatible with these levels. Laboratories with low mean+3SD for deletions may wish to consider results in the 10 – 20% range to be borderline for their own records but they should not be reported to clinicians as positive.

9. The 2005 recommendation that control probes should be used for all deletion probes is no longer considered essential. This recognises that each probe behaves in a unique way and that experience of the probes helps interpretation. In practice many laboratories prefer to use a control probe. In 2005 it was recommended that 13q and p53 should not be used together because of the frequency of double deletions. However, this objection has also been withdrawn.

10. A single experienced analyst is considered adequate to report all abnormalities in the majority of cells from good preparations. However, results should always be checked where there is an equivocal signal pattern, where there are few plasma cells for cIgFISH or where purified plasma cells make up <30% of the total. Smaller labs are recommended to use 2 analysts with a third to check any results with a discrepancy of >5%.

11. Wherever possible 100 cells should be scored. Results are only acceptable from <50 cells where all cells are identical or >75% have the abnormality.

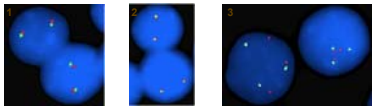
Probes

12. p53, t(4;14) and 13q should be tested in all cases. The t(14;16) also has high priority and many still consider t(11;14) worth doing. Where all these tests are performed there will be extremely few 'normal' 13 results reported incorrectly due to unsuspected near-tetraploidy. However, if an IgH break-apart strategy is used to decide which cases to test for fusion genes then some IgH negative near-tetraploid cases will be missed. The Vysis 5/9/15 probe is recommended to avoid this problem. Alternatively reports should be qualified with regard to the 13q and p53 results.

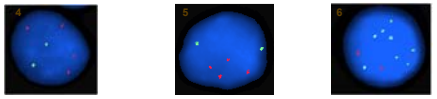
13. Probes to use: **All commonly used probes in 13q14 are acceptable** for 13q deletion which is usually of the whole chromosome.

Fusion strategies must use dual fusion probes that cover a large enough area on the donor chromosome to encompass all breakpoints and allow detection of unbalanced translocations (eg loss of der(14) in t(4;14)). For the t(11;14), the difference between the Vysis standard and Tx probes was not thought to be significant

17p probes should be specifically for p53.



1, 2 & 3 showed normal results for 13q and IgH break-apart, while both 17 centromere and p53 were trisomic.



4, 5 & 6 show CCND1/IgH, FGFR3/IgH and 5/9/15 in the same patient. The former two tests would not have been done by those using IgH break-apart first, and so tetrasomy with effective del(13) would have been missed.

Reporting

14. The method of plasma cell identification should be reported.

The proportion of affected plasma cells should be reported. It is not clear what the correct cut-off level for clinical significance should be. It is suggested that abnormalities in ≤40% cells should be emphasised in the text as being at a low level (although a few laboratories prefer not to report these).

All abnormalities should be expressed as clearly as possible. Thus the workshop did not endorse the use of ISCN.

Fusion probe results (especially for the t(4;14)) should record whether there was a dual or single fusion.

These recommendations should be reviewed yearly by email, with further workshops being held if there is significant controversy. Anyone wishing to be included in the discussions should contact fiona.ross@salisbury.nhs.uk

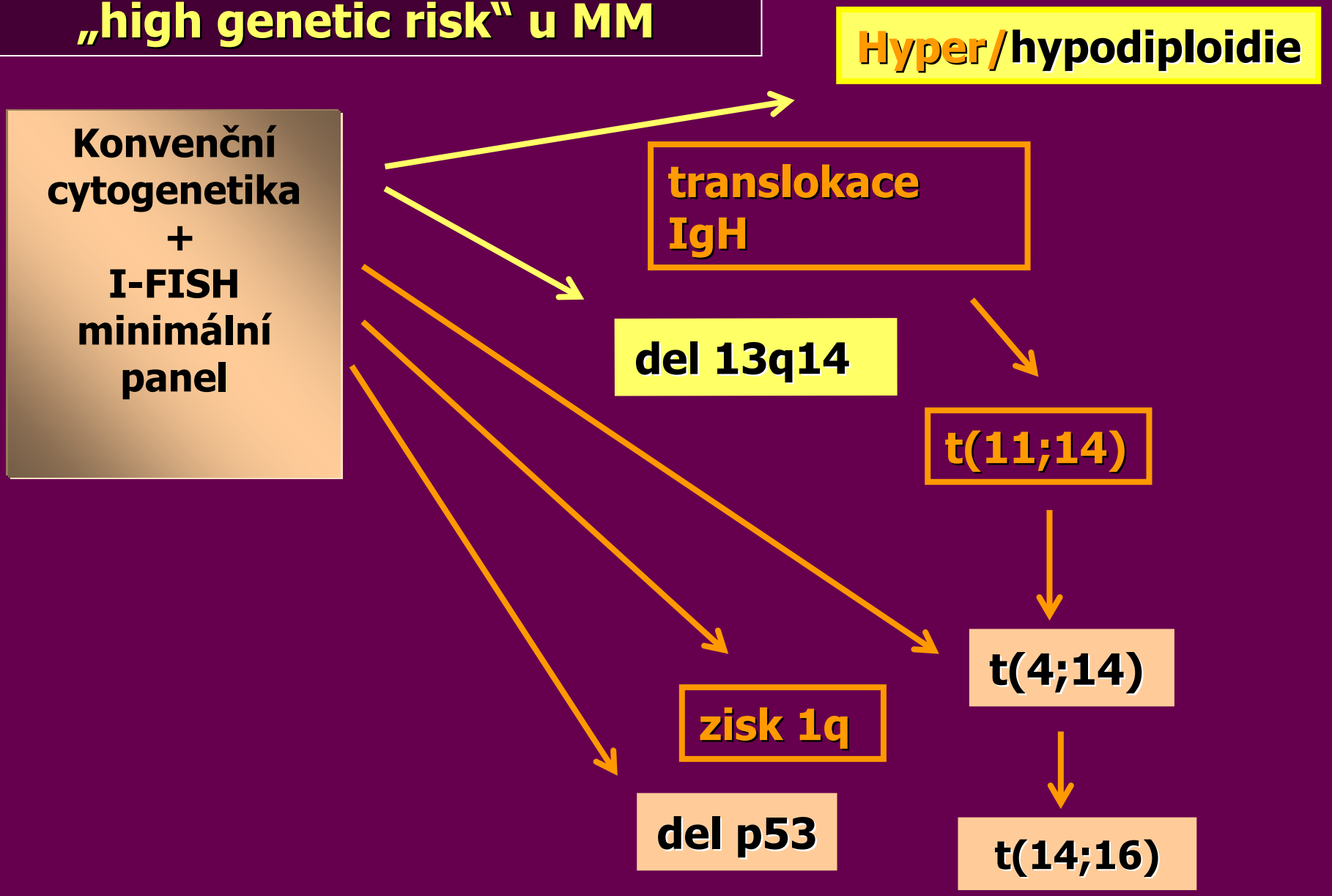
A quality assurance scheme has been set up by Hervé Avet-Loiseau. The first round is underway but anyone wishing to be included in future rounds should contact herve.avetloiseau@chu-nantes.fr

NB the workshop did not address whether or when full cytogenetic analysis should be attempted

Participants

Austria: Johannes Drach (Vienna)
Belgium: Genevieve Ameye (Brussels), Laurence Lespagnard (Brussels), Lucienne Michaux, Heidi Lemmens (Leuven)
Czech Republic: H. Filkova (Brno)
Denmark: Eigil Kjeldsen (Aarhus), Gitte Kerndrup, Anne Grethe Soerensen, Anne Nibe (Odense)
France: Hervé Avet-Loiseau (Nantes)
Germany: Peter Liebisch (Ulm)
Greece: Georgia Bardi, Anna Tasidou (Athens)
Ireland: Patrick Hayden, Johanna Kelly (Dublin)
Italy: Nicolette Testoni, Carolina Terragna (Bologna), Sonia Fabrice, Adele Testi (Milan), Paolo Bernasconi (Pavia), Marina Ruggeri (Turin)
The Netherlands: Clemens Mellink, Simone Snijder (Amsterdam), Birgit Sikkema-Raddatz (Groningen), Arjan Buijs (Utrecht)
Norway: Hong Yan Dai (Trondheim)
Poland: Beata Grygalewicz (Warsaw)
Spain: Juan Cruz Cigudosa (Madrid), Jesus Maria Hernandez Rivas (Salamanca)
Sweden: Bertil Johansson (Lund)
Turkey: Klara Dalva (Ankara)
UK: Mark McKinley (Cardiff), Norman Pratt (Dundee), Sheila O'Connor (Leeds), Angela Douglas (Liverpool),Barbara Czepulkowski, Nicola Foot (London), Nick Telford (Manchester), Jen Beck (Oxford), Laura Chiecchio, Rebecca Protheroe, Fiona Ross (Salisbury), Toon Min, Gareth Morgan (Sutton).
America: Brian Durie (Los Angeles)

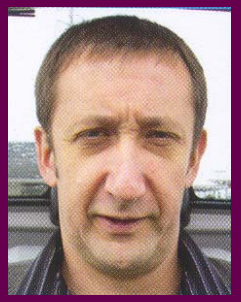
Cytogenetické nálezy a „high genetic risk" u MM



Steward a Fonseca, 2007

EMN a mezinárodní kontrola kvality FISH u MM

Květen – září 2007



- účast 21 evropských laboratoří (prof. Hervé AVET-LOISEAU)
- 10 pacientů s MM
- v balíčku zasláno 100 000 plazmatických buněk ve fixáži
- aberace FISH: del 13q14, del p53, IGH, t(11;14), t(4;14), t(14;16)
- ČR: Praha, Olomouc, Brno

Cíl: porovnat kvalitu FISH vyšetření u MM v rámci laboratoří EMN

MN FISH QC meeting in Nantes, January 22, 2008

Francie, Anglie, Irsko, Holandsko, Belgie, Španělsko, Portugalsko, Řecko, Itálie, Polsko, ČR, Německo, Dánsko, Turecko…

	Patient 6410	Patient 6411	Patient 6412	Patient 6422	Patient 6425	Patient 6426	Patient 6441	Patient 6442	Patient 6444	Patient 6447		
Center A	88%	92%	13%	7%	90%	3%	97%	5%	13%	100%	Abbott-Vysis Rb+D13S319	
Center B	94%	92%	0%	0%	100%	0%	96%	4%	0%	96%	Abbott-Vysis Rb+D13S319	
Center C	96%	92%	2%	4%	97%	6%	93%	2%	3%	87%	Kreatech	
Center D	93%	85%	0%	0%	92%	0%	95%	0%	0%	90%	?	20-40 cells/slide
Center E	86%	95%	8%	9%	90%	5%	91%	5%	8%	93%	Abbott-Vysis	200 cells/slide
Center F	100%	100%	0%	0%	100%	0%	92%	0%	0%	100%	Abbott-Vysis D13S319+13q34	
Center G	95%	100%	5%	3%	100%	1%	97%	1%	0%	98%	Abbott-Vysis Rb	
Center H	97%	97%	0%	5%	98%	3%	96%	4%	4%	95%	Abbott-Vysis D13S319+13q34	
Center I	76%	95%	4%	5%	90%	3%	93%	5%	10%	85%	Abbott-Vysis	200 cells/slide
Center J	95%	98%	0%	0%	94%	0%	99%	0%	0%	99%	Abbott-Vysis D13S319+13q34	
Center K	100%	100%	0%	0%	100%	0%	100%	0%	0%	100%	Home-made	
Center L	100%	97%	4%	7%	100%	55%	100%	8%	5%	99%	Abbott-Vysis D13S319+13q34	
Center M	90%	95%	1%	2%	92%	3%	95%	2%	0%	96%	Abbott-Vysis D13S319+13q34	
Center N	95%	86%	0%	0%	82%	0%	89%	0%	0%	85%	Abbott-Vysis Rb	
Center O	96%	96%	0%	0%	97%	1%	95%	0%	0%	99%	Abbott-Vysis D13S319+ Rb home-made	
Center P	96%	89%	Failure	Failure	99%	6%	89%	0%	1%	92%	Abbott-Vysis D13S319+CEP9	
Center Q	94%	87%	3%	2%	95%	2%	89%	2%	1%	93%	Abbott-Vysis D13S319	
Center R	90%	79%	5%	3%	91%	0%	93%	2%		94%	Abbott-Vysis D13S319+CEP9	
Center S	86%	79%	9%	5%	100%	7%	98%	6%	7%	86%	Abbott-Vysis D13S319+13q34	
Center T	93%	99%	6%	6%	94%	8%	75%	Failure	5%	Failure	Abbott-Vysis Rb	
Center U	95%	97%	3%	2%	96%	2%	94%	4%	3%	96%	Commercial	

Cytogenetika MM a výzkumné projekty

- **Molekulárně cytogenetická analýza plazmatických buněk u pacientů s MM – zahájení studie CMG2008**

  t(4;14), del 17p13, del 13q14, amp 1q21, hyper- a hypodiploidie

- **Úloha chromozomových abnormalit u relabovaných pacientů s MM léčených novými kombinacemi léků (Velcade), Thalidomid)**

- **Zahájení vyšetřování chromozomových abnormalit u pacientů s MGUS**

- **Prognostický význam zisku 1q21 u nově diagnostikovaných pacientů s MM a u pacientů v relapsu léčených novými léky**

- **Genomová profilace pacientů s MM pomocí array-CGH**

URC-CMG
UNIVERSITY RESEARCH CENTRE
CZECH MYELOMA GROUP

Poděkování

- *Centrum nádorové cytogenetiky ÚKBLD VFN a 1.LF UK Praha*
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- J. Rabasová

- *Ústav lékařské genetiky, FN Plzeň*
- M. Hrubá

- *Nemocnice České Budějovice*
- O. Scheinost. M. Lehnerová

Děkuji za pozornost